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CHEMISTRY

A Monte Carlo Investigation of DNA Separation in the Entropic Trap Device

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At present, methods to separate DNA are insufficient for separating large DNA molecules. The entropic trap device fabricated by Craighead and coworkers[1] has some interesting properties that allow large chains to be separated; however, the mechanism by which the device works is not well understood. This study seeks therefore to understand the device's mechanism more thoroughly with a desire to provide the knowledge necessary to optimize the separation of long chains of DNA. The study uses dynamic Monte Carlo simulations on a simple-cubic lattice to optimize the separation of DNA. The results confirm that the code developed recently in Dr. Wang's group correctly simulates DNA electrophoresis. The electrophoretic mobility of DNA in bulk solution was confirmed to be independent of the chain length. The electrophoretic mobility decreases when constrained, but is still independent of chain length. If DNA-wall interactions are added to the model, then the mobility is dependent on the chain length for short chains, but not for chains larger than 30kbp. The entropic trap results showed that the electrophoretic mobility until 60kbp.

INTRODUCTION

Separating DNA by size is important to the biological industry. This is due to the necessity of separating DNA fragments in sequencing a genome or in utilizing the DNA for other purposes. The current standard technique for separating DNA is the Flash Gel system by CAMBREX. This system only claims separation of DNA between 50 bp to 4000 bp. Therefore, a method is still needed to separate long chains efficiently. Other alternative methods do exist,[2,3,4,5] including Pulsed-Field Gel Electrophoresis. Pulsed-field gel electrophoresis however requires long runs, and the use of a high electric field can potentially damage the DNA. The most promising method appears to be separating DNA using entropic traps, particularly the entropic trap device fabricated by Craighead and coworkers.[1] Entropic trapping has been demonstrated to have the capability to separate DNA between 5 kbp and approximately 160 kbp efficiently in a 15 mm channel.[1] The recovery of the DNA is relatively simple also, because the medium used is a buffer solution, which allows the system to be easily integrated into a micro-analytical device to separate DNA. The entropic trap device is composed of a series of open areas (referred to in this paper as wells) and constricted areas (referred to in this paper as channels). The entropic trap device is based on the fact that the chains in the well area have higher entropy; therefore DNA chains are trapped for a period of time that seems to be inversely proportional to the length of the DNA. For this to work effectively, the entropy in the well area must be significantly larger for the range of DNA lengths being examined than that in the constricted region. This implies the requirement that the chain must be allowed to relax while in the well region. This is therefore dependent on both the dimensions of the well, the constricted area, and the applied electric potential. A channel of 1.5 cm long, and 30 μm wide with an applied electrical potential of 0.003V, separated a range of DNA from 1–200 kbp.[7]

Thus, the efficiency, and miniaturization of the device make it a very practical method of separating DNA. Articles by Han and Craighead[1,7,8] sparked interest in the molecular modeling community to understand and optimize the device through experiments. Further studies are required however to understand how the entropic trap array separates DNA molecules. This knowledge may then be used to optimize separation range, the efficiency, and the resolution of the entropic trap device. This will require optimizing the parameters of the device including: the electric field applied, concentrations of the DNA to be separated, and the dimensions of the device.

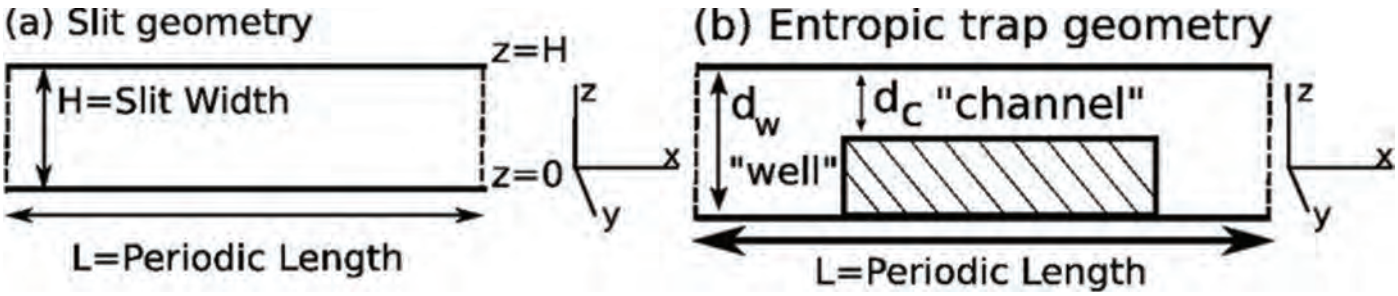

Figure 1: The system is in a 3D lattice, where the x, y, and z directions are given in the figure. The driving electric field is oriented so the negatively charged DNA is driven in the positive x-direction (a) The slit geometry has barriers on z=0, and z=H. Periodic boundary conditions are imposed in the x and y directions. (B) Diagram of a transverse cut of an entropic trap. Periodic Boundary conditions are imposed in the x, and y directions.

Some of these parameters, with regard to the entropic trap device, have been investigated experimentally[1,7,8] and through simulations.[9,10] For example, Han and Craighead found that intermediate electric fields produced the best separation resolution. Furthermore, selectivity is dependent on the number of entropic traps. Other dependencies include the depth of the trap and the constricted region.[7] There are some aspects that are still not well understood, one significant factor is the potential existence of DNA-surface interactions. The DNA-surface interaction has been proposed as another alternative method of separating DNA.[5] Thus, this study will focus on understanding the separation mechanism in an entropic trap device, and the influence of DNA-surface interaction on the separation by using dynamic Monte Carlo simulation algorithm implemented in a program recently developed by Dr. Wang.

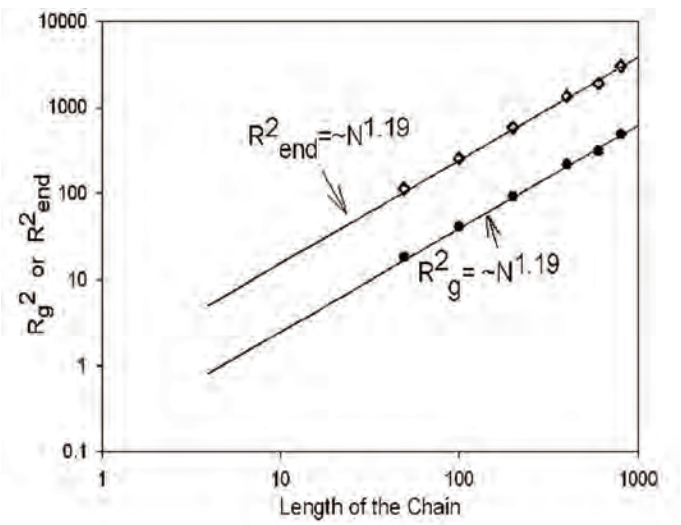

Figure 2: The open diamonds signify the end-to-end Vector values, and the closed circles represent the radii of gyration for various chain lengths. The power function that fits the radius of gyration data is y= $(0.156\pm0.07)N^{1.19\pm0.07}$. The power function that fits the end-to-end vector data is y= $(0.07\pm0.5)N^{1.19\pm0.08}$.

METHODS/THEORY

Simulation System: Molecules

DNA movements are modeled as self-avoiding walks on a simple cubic lattice. Each DNA molecule is represented by N connected beads, where N represents the length of the DNA chains. Each bead is assumed to represent 150 bp, and the distance between two connected beads is about 50 nm. During the simulations, no two beads are allowed to overlap the same lattice sites. The empty sites are considered to be occupied by solvent molecules. They are implicit in the model; therefore, the hydrodynamic interactions are not considered in the simulations. The total number of DNA chains was kept sufficiently low to maintain a volume fraction of DNA solution at 0.05 to 0.02. The concentration was measured by calculating the volume fraction. This was defined by dividing the total number of beads by the total number of points on the lattice.

Geometry of the system

In this study four types of systems were studied, a bulk solution, a slit with and without interactions, and an entropic trap system. The bulk solution was simulated in a 3-dimensional cubic lattice with periodic boundary conditions applied

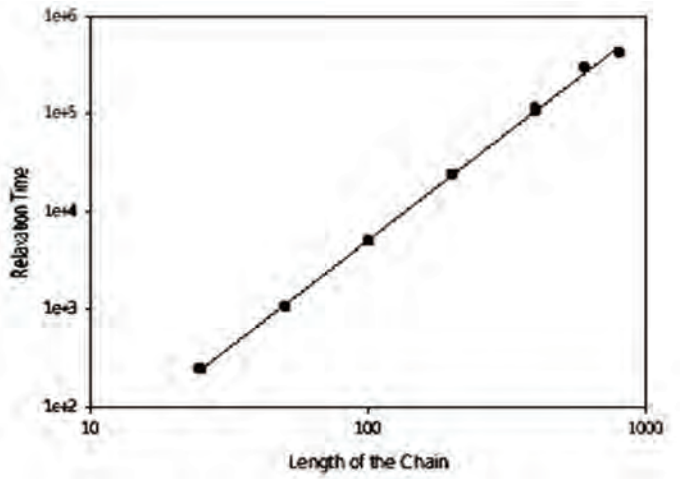

Figure 3: The relaxation time as a function of the chain length. The points were fitted to a power function to give: y=$(0.218\pm0.03)N^{2.19\pm0.03}$.

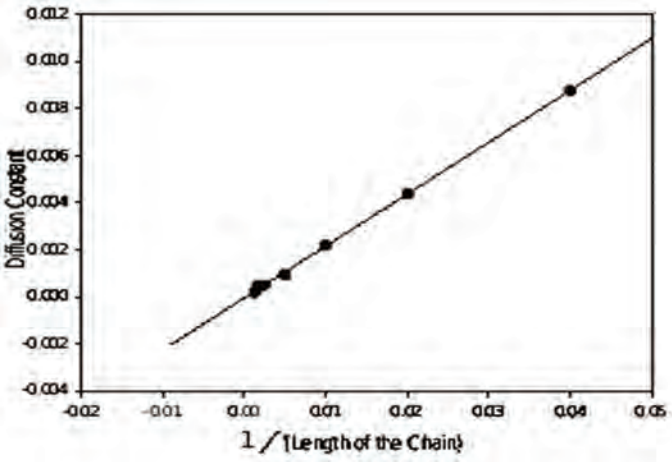

Figure 4: Diffusion Constant in a bulk solution as a function of the inverse length of the chain. The linear fit to this curve is: $y=(0.22\pm0.002)x^{-(5\pm1)E-5}$.

performed with this geometry one with a surface interaction of ε_w =0, and the other with a surface interaction of ε_w =-1.0. The runs in the slit geometry were also kept at a volume fraction of approximately 0.02 in the case of the unconstrained chains, and 0.05 for the constrained chains.

The entropic trap device is shown in Figure 1. The device is modeled by constructing a lattice such that the walls are considered occupied. Periodic boundary conditions are imposed in the x and y directions. The well region must have a dw larger than the radius of gyration (R_g) to allow the chain to relax. The constricted region must have a d_C smaller than the R_g value. In the case of this study, d_C=20, d_w=100, and L=400. A potential difference was applied along the x direction across the simulation box. The walls of the channel were considered perfect insulators and the Laplace Equation was solved for the geometry in Figure 1 to obtain the local potential for each lattice site in the entropic trap case. In the case of the bulk simulation, and the slit simulation, a potential difference was also applied in the x direction across the simulation box. The electric field created by the potential difference in these two cases are homogenous and is simply given by $\varepsilon_w = V/L_x$, where V is the potential difference across the box. All simulations are reported in terms of this applied electric field, which in the current study is varied from 0.001 to 0.1. In the case of entropic trap, the local electric field is not homogenous, but is determined by the solution to the Laplace equation in that geometry. This article focuses on a device fabricated by Han et al.[6] and investigates further the methods by which this device works by using Monte Carlo simulations.

Movement of Chains

The algorithm used to move the chain randomly is very similar to the Crab-Kovac Model.[11] This model allows a bead to make elementary one-bead moves to an empty space, and a 90°-crankshaft motion is allowed to obtain correct dynamics for the Self-Avoiding Walk (SAW) chain. The one-bead move allows a move to a neighboring site while preserving chain connectivity. The 90°-crankshaft motion is a two bead move, where two bead move together rotating 90° around a central axis.

Static Properties Measured

The radius of gyration was calculated according to Equation 1, where N is the number of beads on a chain in the system, and rk is the position of kth bead, and r_{mean} is the position of the center of mass of a chain. The bracket stands for the ensemble average. The final reported Rg value is also averaged over all the chains in the system.

$$R_g^2 \equiv \frac{1}{N}\left\langle \sum_{k=1}^{N} (\vec{r}_k - \vec{r}_{mean})^2 \right\rangle$$

The end-to-end distance was calculated in a similar manner according to Equation 2. In this case the positions of each chain's first and last beads are subtracted; rfirst and rlast are the positions of the first and last beads of a chain.

$$R_{end}^2 \equiv \left\langle \sum (\vec{r}_{first} - \vec{r}_{last})^2 \right\rangle$$

Dynamic Properties in an Equilibrated System

The diffusion constant was determined by graphing the square of the distance the center of mass moved with respect to time and taking the slope to be the diffusion constant. Equation 3 gives the autocorrelation function of a vector (i.e. end-to-end distance vector or the Rouse vector with mode p=1).

$$C(t) = \frac{\langle R(t) \cdot R(0) \rangle}{\langle R^2 \rangle} = e^{\frac{-t}{\tau}}$$

(Eq. 3)

The correlation function is the dot product of a vector at time t with the vector at time t=0, and then divided by its amplitude squared. The autocorrelation function determined is then fitted to the simple exponential decay to obtain the relaxation time τ. To determine the relaxation time, a simulation time of approximately two hundred times the approximate relaxation time was used. The chains were also allowed to equilibrate for a minimum of one to two times the relaxation time.

Dynamic Properties in a Non-Equilibrated System

The drift velocity is taken to be the distance traveled by the chain's center of mass during a Monte Carlo step. The position of the center of mass of the chain was therefore graphed with respect to time. The slope was then taken as the drift velocity, v. The electrophoretic mobility, μ_0, was then calculated using $v=\mu_0\varepsilon$, where ε is the electric field applied.

To determine the electrophoretic mobility of a chain, a simulation time of one to five times the relaxation time was used. Then, the electrophoretic mobility for a length of chain was determined by varying the electric field a minimum of five times, ranging from 0.001 to 0.1, and calculating the drift

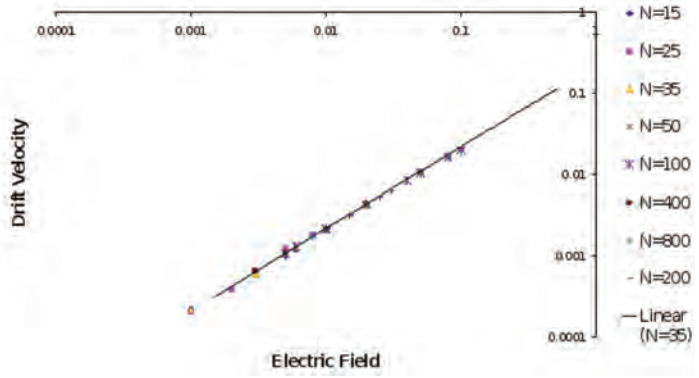

Figure 5: The electrical field plotted against the drift velocity in a bulk solution. The electrophoretic mobility of a chain is the slope of the drift velocity graph as a function of the applied electrical field, ε . All of the points are close to a single line. Thus, in bulk solution the electrophoretic mobility is independent of chain length.

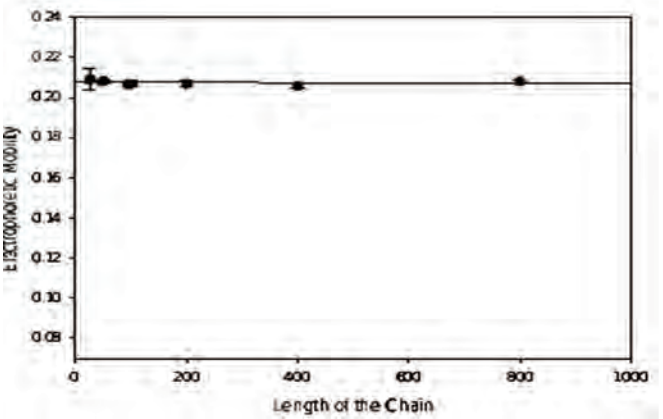

Figure 6: The electrophoretic mobility of chains in a slit of H=20, with a surface interaction of ε_w = 0.0.

velocity for each electric field. No electric fields greater than 0.1 were used for any of the simulations.

RESULTS AND DISCUSSION

a) Results in a bulk solution with no electric field

We first performed simulations for dilute bulk polymer solutions with no electric field applied and determined static and dynamic properties for chains with different lengths. These are used to verify the simulation model. The DNA are modeled as self-avoiding walks, therefore their radius of gyration and the end-to-end distance should obey the following relation, $R^2 = N^{2\nu}$, where ν is called the Flory's exponent. The Flory exponent's accepted value is ν =0.58813. The Flory constants calculated from the data given in Figure 2 were 0.59±0.03 from the radius of gyration and 0.59±0.03 from the end-to-end distance, in a good agreement with known theoretical value.

The relaxation time and the diffusion coefficient of the chain are also obtained. Their dependence on the chain length are also known theoretically. The relaxation time t should obey the relationship, $\tau = \alpha N^{1+2\nu}$. The simulation data is presented in Figure 3.

The exponent determined from the data is 2.19±0.03, in good agreement with the expected value of $1+2\nu$ =2.18, if taking ν as 0.59. The diffusion constant should scale inversely proportional to the chain length as follows, $D = \alpha N^{-1}$.

Simulation data are presented in Figure 4, where a linear relationship between D and 1/N was well-observed. All the results are very close to expected values for a SAW in a 3D lattice. Thus, the program used for this simulation properly models a SAW chain

b) Electrophoretic mobility in bulk solution

Another important characteristic of DNA electrophoresis is that its electrophoretic mobility in dilute bulk solution is independent of its length when DNA fragment has greater than 100bp. This is also the reason that for separation purpose DNA electrophoresis is performed in a gel or in some medium that can hinder the motion of the chain. To model the DNA electrophoresis properly, the simulation should reproduce the result where the bulk electrophoretic mobility is independent of the chain length.

To determine the bulk electrophoretic mobility, drift velocity was determined using at least five different applied electric fields, ε from 0.001 to 0.1 range. Figure 5 demonstrates that the simulation is correct by confirming the fact that the electrophoretic mobility in a bulk solution is independent of chain length. The electrophoretic mobility was determined by taking the slope of the graph in Figure 5. Thus, the electrophoretic mobility is constant with respect to chain lengths, and with electric fields below 0.01.

c) Electrophoretic mobility in a slit

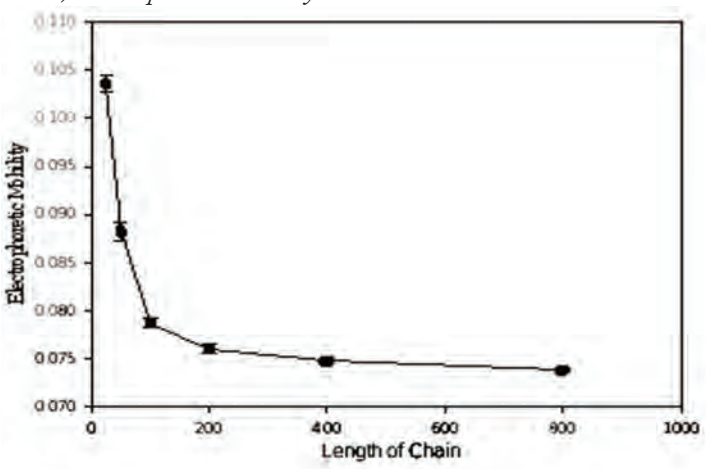

Figure 7: The electrophoretic mobility of chains in a slit of H=20, with a surface interaction of ε_w = -1.0.

Two sets of simulations are performed to determine the

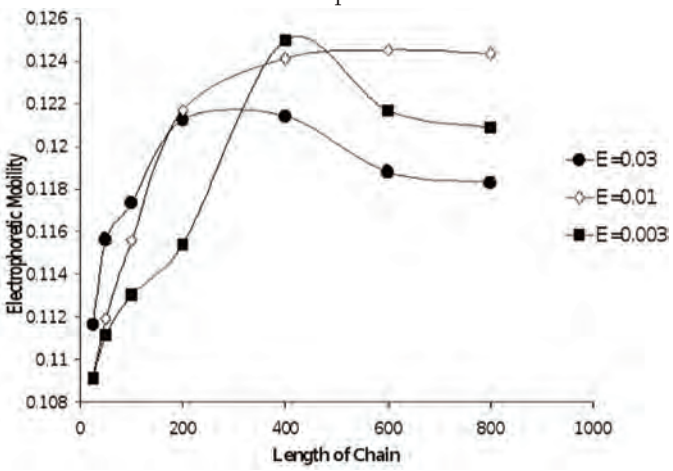

Figure 8: Electrophoretic mobility as it varies with the length of the chain in the entropic trap array. The electrophoretic mobility increases as the chain length increases.

electrophoretic mobility of chains in a slit with H =20. In one set the surface has no interaction and on the other set the surface is adsorptive with ε_w=-1.0. Figure 6 shows that constrictions alone due to the slit geometry do not alter the electrophoretic mobility. The electrophoretic mobility of the chains still remain very close that in the bulk solution, around 0.209± 0.003. If interactions are added however, then Figure 7 shows that the electrophoretic mobility is not constant. Although, chains having a length larger than 400 have an electrophoretic mobility that is essentially constant. If each bead therefore is assumed to be equivalent to 150bp, then one may say that the slit with interactions is capable of separating DNA up to a length of 30,000bp.

d) Entropic Trap Array

As can be seen in Figure 8, the electrophoretic mobility in an entropic trap array is directly related to the chain length. The mechanism by which this device works has not been investigated however in this study at the present time. However, in general these results do confirm the results from prior experimental studies up to 60kbp.[1,7,8] The reason for the deviation compared to the expected results after 60kbp is unknown. These curves however require further characterization and study to draw any definite conclusions.

CONCLUSIONS

It can be concluded from these results that the simulation correctly models DNA electrophoresis. In a bulk dilute solution, in the absence of any obstacles or constriction, the electrophoretic mobility is independent of the chain length, a known behavior for DNA electrophoresis. When a chain is in a constrained environment, it moves slower than in a non-constrained environment, but it could not be used to separate large chains effectively. A slit with DNA-wall interactions separates DNA with length below 400 effectively, but larger chains are not effectively separated. In the entropic trap device, the simulations confirmed that long chains do move faster with the device. In the future, more studies will be completed concerning DNA-wall interactions, by studying the effects of the strength of the interactions of a bead with the surface. Also, the entropic trap mechanism will be explored further by performing more calculations on the entropic trap.

REFERENCES

1. Han, J.; Craighead H.G. Science 2000, 288, 1026
2. Viovy ,J. Rev. of Mod. Phys.2000,72,813
3. Slater, G.W.;Kenward, M.;McCormick, L.C.;Gauthier,M.G. Cur. Op. in Biotech. 2003,14,58
4. Luo H.; Gersappe D. Electrophoresis 2002,23,2690
5. Pernodet, N.; Samuilov, V.; Shin, K.; Sokolov, J.; Rafailovich, M. H.; Gersappe, D.; Chu, B. Phys. Rev. Let. 2000,85,5651
6. Ashton,R.;Padala,C., Kane, R.S. Cur. Op. in Biotech. 2003,14,497
7. Han, J.;Craighead,H.G. Anal. Chem. 2002,74,394
8. Han, J.; Turner, S.W.; Craighead,H.G. Phys. Rev. Let. 1999,83,1688
9. Tessier,F.;Labrie,J.;Slater,G.W. Macromolecules 2002,35,4791
10. Streek, M; Schmid,F.;Duong,T.T.; Ros, A J. of Biotech. 2004,112,78
11. Naghizadeh, J.; Kovac, J. Phys. Rev. B 1986,34,1984
12. Frenkel, D.; Smit, B Understanding Molecular Simulation: From Algorithms to Application; Computational Sciences Series; Academic Press: San Diego, CA, 2002; Vol. 1, pp 27-32.
13. de Gennes, P.-G. Scaling Concepts in Polymer Physics, Ithaca, Cornell University Press, 1979.