The Effect of Knotting in Translocation of a Confined Polymer through a Hole

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Monte Carlo simulation data were used to examine the effect of knotting in the translocation of a confined polymer through a hole. Since our polymers are open chains, two operational definitions were used to determine knotting, which is defined only for closed chains. The probability for knot formation inside a spherical shell was found to decrease from (14.7 ± 0.3)% to (13.3 ± 0.9)% using a method without chain reduction and from (8.4 ± 0.3)% to (4.7 ± 0.5)% using a method that reduced and simplified the chain before knot determination. When polymers were allowed to exit the sphere of confinement through a small hole in the shell (such that only one monomer at a time could escape) the full time to escape was found to be $6.4 \times 10^7 \pm 6.1 \times 10^7$ Monte Carlo steps for configurations that began unknotted, and $8.8 \times 10^7 \pm 2.7 \times 10^7$ Monte Carlo steps for configurations that began with one trefoil knot. Configurations that began with a knot unknotted very quickly, and at a qualitative level there appears to be no substantial difference between ejection in knotted and unknotted initial configurations. However a larger sample size may reveal knotting effects to be rare but important.

INTRODUCTION

For a parasite that is not technically even alive, viruses are capable of performing amazing biological feats. They feed DNA or RNA into a small capsid, producing forces of up to 50pN — around 10 times the force applied by cellular kinesin proteins [1, 2]. For a typical phage virus, a genome that measures some 13.6μm when fully extended is packaged into a virus approximately 1μm in diameter [4]. The density of such packaged DNA in a virus is around five times that of metaphase chromatin in human cells [4].

The study of virus structure is notoriously difficult given their small size and hard protein shells. Recently, many advances have been made regarding the details of DNA packing mechanisms and forces in bacteriophage (i.e. phage) viruses including packing forces, proteins involved (including motors), and final packed conformations [1, 3, 4]. However, significantly less is know about the injection of such packed DNA from the virus into the host cell. Is it driven simply by the pressure of the DNA in the virus capsid, or are there other sources of energy? Can the DNA be entangled or knotted, and how does this affect ejection?

Approached from a more physical perspective, the problem of virus DNA ejection can be distilled to the translocation of a confined polymer through a hole. Muthukumar has studied such translocation events using Monte Carlo simulation and suggests that for a beads-on-a-string polymer there is a free energy barrier between the fully-confined and fully-ejected state, leading to multiple reversed partial ejections before a final full ejection of the polymer [5]. This study, however, did not address the complication of entanglement and knotting in the polymer [5].

A study by Arsuaga *et al.* found that between 50% and 90% of P4 phage DNA was knotted, and simulation studies reveal increased knotting in confined conditions such as in a virus capsid [6, 7]. Additionally, in mutant P4 phage, for which both ends of DNA were free, knotting probability dramatically increased [6].

Since knots appear to be so common in packaged viral DNA, we investigated the effect of such knots on the translocation of a confined polymer through a hole. First we examined knotting probabilities in a confined sphere both with one end of the polymer fixed, and with both ends mobile. After that, several simulations of polymer escape through a small hole were run, both for initially knotted and unknotted configurations. The probability for knot formation was found to decrease from (14.7 ± 0.3)% to (13.3 ± 0.9)% without chain reduction before knot determination and from (8.4 ± 0.3)% to (4.7 ± 0.5)% using a method that reduced and simplified the chain before knot determination. The full time to escape was found to be $6.4 \times 10^7 \pm 6.1 \times 10^7$ Monte Carlo steps for configurations that began unknotted, and $8.8 \times 10^7 \pm 2.7 \times 10^7$ Monte Carlo steps for configurations that began with one trefoil knot.

METHODS

We used Monte Carlo simulation to study polymer translocation through a small hole. The polymer was modelled as a sequence of beads in three dimensional space, as described by Virnau *et al.* [7]. Two monomers of separation r interact by the truncated and shifted Lennard-Jones potential [7]:

$$V_{\mathrm{LJ}}(r) = \begin{cases} 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} + \frac{127}{16384}\right], & r < r_c \\ 0, & r \geq r_c \end{cases} \quad (1)$$

Adjacent beads of the polymer are connected with finitely-extensible nonlinear elastic (FENE) springs, de-

scribed by the potential [7]:

$$V_{\text{FENE}}(r) = -33.75 \cdot \epsilon \cdot \ln\left[1 - \left(\frac{r}{1.5\sigma}\right)^2\right] \qquad (2)$$

Where ϵ defines the depth of the potential, σ defines its width, and $r_c = 2\sqrt[6]{2}\sigma$ is the cut-off distance. Configurations were confined in a hard-walled sphere of radius 4.5σ with sphere cantered restricted from entering a shell of 0.45σ. All polymers contained 200 monomer subunits, and all simulations took place at T = $4.98\frac{\epsilon}{k_b}$, which corresponds to 150% of the Theta temperature of the model.

Determining Knot Probabilities

Before simulation of polymer translocation, the probability of knotting in the confined volume was determined both for polymers with two free ends and for polymers with one end fixed on the confinement shell at coordinates (0, 0, 4.5σ). In these simulations the shell was a complete sphere (no opening for translocation). For polymers with both ends free, slithering snakes steps as well as local Monte Carlo moves were allowed. With one end fixed, slithering snake moves were impossible, so only local moves were allowed. 10,200 separate conformations were evolved to determine knotting probabilities for polymers with both ends free. 1,520 conformation were evolved to determine probabilities for polymers with one end fixed. Mathematically, knots can only exit in closed loops. However, a systematic procedure to close an open loop can be applied. Two such procedures were used. In the first the open ends were connected to infinity with straight lines, and in the second a reduction scheme was applied before connection. See Virnau *et al.* for further discussion [7].

Translocation

After knotting probabilities were determined, both knotted and unknotted starting conformation were chosen from the conformations created with one end fixed. Fixed-end conformation were selected to mimic viruses, which anchor one end of their DNA [8, 9]. All conformations with knots that were selected for further evolution contained exactly one trefoil knot (with three crossing — the most simple knot possible). The sphere of confinement was modified to have a hole of 0.75 on the z axis at (0, 0, 4.5σ) such that one monomer at a time could exit the sphere through the hole. The fixed end was allowed to move freely and exit through the hole. The starting conformations were then evolved using local Monte Carlo steps until the polymer was completely free of the sphere. Data, including the occurrence of knots, the radius of gyration of the polymer, and the number of monomers inside and outside the sphere, were collected every 2,500 Monte Carlo steps. Ten translocation events were simulated from unknotted start conformations and 11 with knotted start conformations.

Configuration	% Knots 1	% Knots 2
Both Ends Free	14.7 ± 0.3	8.4 ± 0.3
One End Fixed	13.3 ± 0.9	4.7 ± 0.5

TABLE I: Results for knotting probabilities in polymers with and without fixed end. Probabilities were determined from 10,200 free end configurations and 1,520 configurations with one end fixed. Method two used simplification and reduction of the polymer before determining knotting, while method one did not as described in [7]

RESULTS AND DISCUSSION

Results for knotting probabilities are shown in Table I. Using both methods of knot determination, the knotting probability decreased for conformations generated with one end of the polymer fixed to the spherical boundary. This is in qualitative agreement with Arsuaga *et al.* however our computational results give a much smaller probability of knotting than their experimental results which ranged from 50% to 90% [6]. This difference could be due to simplifications in our model (no bending stiffness or charge, and only 200 monomers) or to excess knotting that may occur after release from the capsid in the Arsuaga *et al.* experiment. Differing temperatures may also have played a role in the different probabilities.

The results of representative translocation events are shown in Figure 1. Due to small sample size (only 21 translocations in total) much of the results are qualitative in nature. Looking at Figure 1 suggests that there is no free energy barrier to polymer ejection as ejection initially proceeds very quickly with little to no reversal of direction, only stalling later when ejection was nearly complete. This contradicts the results of Muthukumar, and may be due to differences in our simulations — most notably, Muthukumar's monomers are charged while ours are not [5].

Comparing the process of polymer escape between knotted and unknotted initial conformations is difficult due to the limited sample size. The average escape time was $6.4 \times 10^7 \pm 6.1 \times 10^7$ Monte Carlo steps for unknotted start configurations and $8.8 \times 10^7 \pm 2.7 \times 10^7$ Monte Carlo steps for knotted start configurations. Of note, is the fact that nearly all the conformations that started knotted became unknotted very early in the ejection process. Since unknotting is much more probable than knotting in a given Monte Carlo step, finding conformations with knots in the later stages of ejection would require many more trials. Ideally, many trials could be compiled

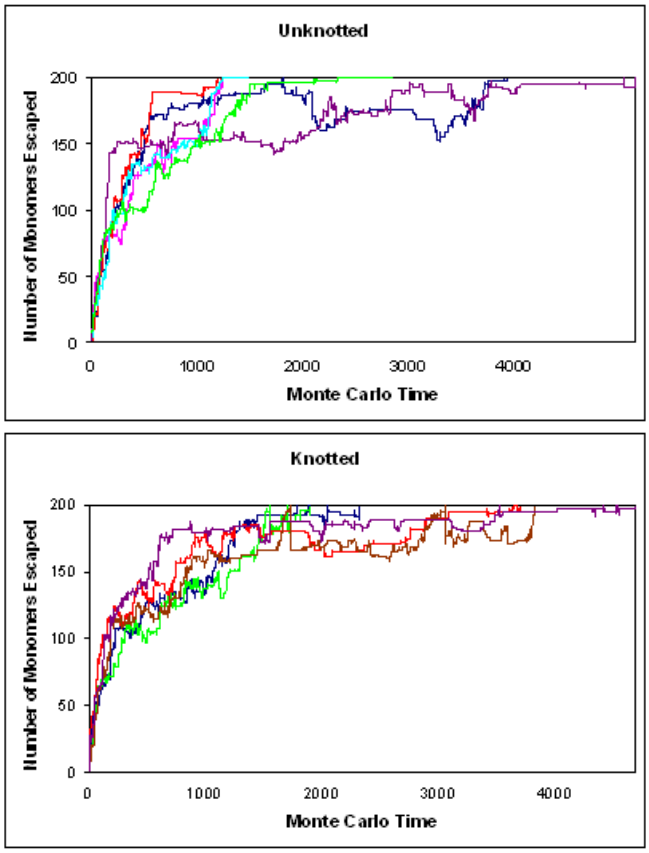

FIG. 1: Representative translocation events for knotted and unknotted initial conformations. Time steps represent 2,500 Monte Carlo steps.

to create a histogram of Probability vs. Time of Full Ejection. Knotting in later stages might be revealed in a modified probability distribution for later times. With a larger sample size average plots of Number of Escaped Monomers vs. Monte Carlo Time could be compared to each other and to theoretical predictions.

Since the polymer model for our study did not include either charge or bending stiffness, it is only a first step towards simulating ejection of DNA from a virus. To more realistically model biological phenomenon, electrostatic interactions should be included. Also, to simulate double-stranded DNA, a bending stiffness could be included. Previous simulation studies suggest that charge and stiffness are significant to DNA packing inside a virus, but did not investigate their effect on DNA ejection [10, 11]. Additionally, many viruses are icosahedral, therefore it would be beneficial to consider an icosahedral outer boundary.

CONCLUSION

The probability for knot formation in confinement was found to decrease from $(14.7 \pm 0.3)\%$ to $(13.3 \pm 0.9)\%$ without chain reduction before knot determination and from $(8.44 \pm 0.3)\%$ to $(4.7 \pm 0.5)\%$ using a method that reduced and simplified the chain before knot determination. The full time to escape was found to be $6.4 \times 10^7 \pm 6.1 \times 10^7$ Monte Carlo steps for configurations that began unknotted, and $8.8 \times 10^7 \pm 2.7 \times 10^7$ Monte Carlo steps for configurations that began with one trefoil knot. Based on the preliminary results of our study, it seems that knotting in DNA does not affect ejection of DNA from a virus. However, larger sample sizes may reveal that, while rare, knotting is important in ejection, and more realistic simulations may yield different results.

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