DNA Internal Motion Likely Accelerates Protein Target Search in a Packed Nucleoid

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ABSTRACT Transcription factors must diffuse through densely packed and coiled DNA to find their binding sites. Using a coarse-grained model of DNA and lac repressor (LacI) in the Escherichia coli nucleoid, simulations were performed to examine how LacI diffuses in such a space. Despite the canonical picture of LacI diffusing rather freely, in reality the DNA is densely packed, is not rigid but highly mobile, and the dynamics of DNA dictates to a great extent the LacI motion. A possibly better picture of unbound LacI motion is that of gated diffusion, where DNA confines LacI in a cage, but LacI can move between cages when hindering DNA strands move out of the way. Three-dimensional diffusion constants for unbound LacI computed from simulations closely match those for unbound LacI in vivo reported in the literature. The internal motions of DNA appear to be governed by strong internal forces arising from being crowded into the small space of the nucleoid. A consequence of the DNA internal motion is that protein target search may be accelerated.

INTRODUCTION

Proteins find their binding sites on DNA by the process of diffusion. It is firmly believed that this diffusion consists of one-dimensional (1D) sliding and hopping motions along DNA as well as relatively free three-dimensional (3D) diffusion within the nuclear space (see, e.g., (1)). This theory of facilitated diffusion was developed to help explain how the association rate of the lac repressor (LacI) with its operon is two orders-of-magnitude higher than what would be expected by 3D diffusion alone (2,3). By reducing the dimensionality of the search by incorporating 1D diffusion, the association rate can be accelerated.

In this article, we seek to address how 3D diffusion of LacI occurs within the packed nucleoid, the condition in vivo of DNA being coiled and densely occupying a small space. For Escherichia coli, the average distance between DNA strands is 6.4 nm (4,5), creating an average spacing between strands less than the diameter of large transcription factors such as LacI. To understand how LacI diffuses in such an environment, we use computer models that allow for DNA strands to be flexible and in motion.

Another issue that we seek to address is the potential slowdown of the association rate for facilitated diffusion compared to 3D diffusion alone given the findings that LacI spends ~90% of the time nonspecifically bound and diffusing in 1D along DNA, and that the 1D diffusion constant is observed to be ~65 times smaller than the 3D diffusion constant (6). In particular, 1D diffusion would seem only to accelerate the search in a dilute DNA solution, such as that originally used by Riggs et al. (2) and in other early in vitro experiments (e.g., see the reviews in (7–9)). We hypothesize that DNA internal motions accelerate the target search in vivo despite slow 1D diffusion of proteins bound nonspecifically to DNA.

Many studies of facilitated diffusion use a single, straight, possibly rigid segment of DNA to investigate, for example, aspects of protein rotation-coupled sliding (e.g., (10–14)). Other studies focus on the effect that DNA conformation may have on facilitated diffusion (e.g., (15,16)). Still other studies consider the effect of crowding, from proteins either diffusing or bound on DNA (e.g., (17–20)), or less commonly, from tightly packed DNA (e.g., (21)). There are few studies where DNA diffusive motions are considered (e.g., (16,22)), and this is the focus of the study here. We note that in previous work (13), we have simulated a flexible DNA chain with its end points restrained, and investigated the effects of hydrodynamic interactions on protein rotation-coupled sliding. With Brownian dynamics (BD) simulations, it was shown that DNA motions could at least double the 1D diffusion constant. In contrast to this previous
work, our study focuses on understanding protein diffusion through a packed DNA globule where DNA motion is not artificially restrained, and necessarily uses a much coarser model of protein and DNA.

One view of protein target search in the nucleoid is that the spacing between DNA segments is sufficiently great so that the effect of DNA diffusion on LacI 3D diffusion is minor. At the other extreme, DNA is densely packed so that its motion actually dictates the global protein motion. Clearly, which regime a protein falls in depends not only on the DNA concentration but the diameter of the protein relative to the average intersegment spacing of the DNA. We constructed a simple, idealized model of DNA and LacI packed in the E. coli nucleoid at correct physiological concentrations and performed coarse-grained dynamical simulations. We also consider the effect of hydrodynamic interactions (HI) in our models using the Stokesian dynamics (SD) method (23).

A typical E. coli K-12 cell has a nucleoid volume of 0.14 μm³, containing an average of 2.3 genomes, each 4,639,221 basepairs long (4,5,24). We model DNA as a string of touching beads, each with radius 1.59 nm, a value that has been used in earlier studies to reproduce the hydrodynamic properties of DNA (25). Thus, each bead represents 9.35 basepairs and the resulting volume fraction of DNA is ~13%. We note that although this volume fraction is not known precisely, other researchers have quoted a range of 10–20% (26). Stretching and bending potentials are also used between beads, with the bending constant chosen to reproduce the persistence length of DNA. DNA beads are negatively charged and Debye-Hückel potentials are used to model these interactions. This model of DNA closely matches the model of Jian et al. (27). Our coarse-grained models are much coarser than other models that have been used to understand facilitated diffusion when nucleoid packing was not considered.

The persistence length of DNA is 50 nm, but this would imply a DNA volume much larger than what would fit within the nucleoid. DNA is highly compacted in vivo, due to macromolecular crowding, DNA coiling by nucleoid-associated proteins, and other effects (5). To construct an initial DNA configuration for simulation, we first generated a continuous chain of beads (the first bead is connected to the last bead) arranged compactly with right-angle turns of the DNA not occurring at less than twice the persistence length (the Kuhn length). LacI is a tetramer with total molecular mass of 154,520 Da and is modeled by a sphere of radius 4.4 nm, a value comparable to the hydrodynamic radius of other proteins with similar weight (28).

The model DNA in the above geometric arrangement and a single LacI sphere are placed inside a periodic simulation box. Dynamical simulations without HI are then performed while gradually shrinking the simulation box until the target volume fraction is achieved. This naturally coils and compacts the DNA into a small volume and gives the initial simulation configuration.

Fig. 1 shows the initial configuration of a LacI-DNA system in a periodic simulation box. In this system, 6912 DNA beads are used (~64,627 basepairs) with one LacI sphere. The simulation box is reduced from an initial width of 556.5 nm to a final width of 96.3 nm. In addition to this large system, we also performed simulations with a much smaller system of 256 DNA beads (27 times smaller) and one LacI sphere reduced to a 32.1-nm width simulation box. Fig. 1, based on realistic nucleoid and DNA dimensions mentioned above for E. coli (4,5), shows that DNA exists in a much more packed environment than is typically imagined. Although the 13% volume fraction of DNA we estimate in the nucleoid is <20–40% volume fraction of macromolecules in the cytosol (28,29), we expect DNA packing to play a complex role in diffusion in the nucleoid just as macromolecular crowding affects diffusion in the cytosol.

MATERIALS AND METHODS

We use a model of DNA that closely matches the model of Jian et al. (27). Stretch, bend, and Debye-Hückel potentials are used. The stretch potential $\delta/2(x^2 - 2a^2)$ for two adjacent DNA beads used force constant $k = 100 k_B T a^2$, where $a$ is the center-to-center distance between the beads.

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**FIGURE 1** Given here is the initial configuration of the LacI-DNA system with 6913 total particles, with DNA beads drawn as a chain. (A) The system is drawn within its periodic simulation box. (B) The same system is drawn, showing that the DNA beads form a continuous chain. The portions of DNA outside the box also exist as periodic images within the box, which are not drawn. (C) Both previous figures are superimposed. (D) Portions of the DNA system have been cut away to show the LacI sphere crowded in its DNA environment. To see this figure in color, go online.
and \( a \) is the DNA bead radius. The bending potential \((g/2)\theta^2\) for an angle \( \theta \) defined by three beads used rigidity constant \( g = 14.8 \ k_BT \) was selected using long simulations in infinitely dilute conditions to match a DNA persistence length of 50 nm.

Electrostatic interactions in the nucleoid, which are screened by ions, are modeled by the Debye-Hückel potential. In all simulations, DNA beads are charged. The negative charge per DNA bead assumes a DNA charge distribution of \(0.243 \ e/\AA\), where \( e \) is the electron charge \((27)\). A positive charge of twice the magnitude of a DNA bead charge is also placed on the surface of the LacI sphere in simulations of LacI 1D hopping. Following the model used in Jian et al. \((27,30)\), we use a Debye length of \( \lambda_D = 3.07 \) nm, corresponding to a salt concentration of 0.01 M. Multivalent ions were not considered. For the small DNA model, interactions between all particles in the simulation box (or between their nearest periodic images) are computed. For the large DNA model, a cutoff of eight DNA bead radii \((12.72 \) nm\) was used, and applied using cell lists for computational efficiency. However, electrostatic Debye-Hückel interactions are excluded between adjacent and next adjacent DNA beads. We note that, for the large models without HI, computing the Debye-Hückel interactions constitutes the majority of the computational cost.

BD simulations \((31)\) without HI, i.e., using the free-draining (FD) approximation, utilize a steric potential between overlapping particles as follows:

\[
V_s = \begin{cases} \frac{1}{2}k_B(x' - 2)^2 & \text{if } x' < 2 \\ 0 & \text{if } x' \geq 2, \end{cases}
\]

where \(x' = 2x(a_i + a_j)\) is the normalized distance between two particles, \(x\) is the actual distance, and \(a_i\) and \(a_j\) are the radii of the two particles. A value of \(k_B = 100 \ k_BT\) was used, matching the DNA stretching force constant.

BD simulations that model HI use a mobility matrix (proportional to a diffusion matrix) that describes such interactions between pairs of particles. It is common to choose a mobility matrix, \(M^+\), whose entries are defined by the Rotne-Prager-Yamakawa tensor \((32,33)\). However, this BD method is not suitable for short-range interactions (relative to the size of the particles) such as those in vivo, and thus we instead use SD simulations \((23)\). Here, the inverse of the mobility matrix, called a “resistance” matrix, is modeled, and a common choice is to use \((M^+)^{-1} + R_{\text{lab}}\). The second term models short-range HI, called “lubrication forces”, and its entries are computed by formulae in Jeffrey and Onishi \((34)\). In particular, these formulae use scalar lubrication functions for equal and also differently sized spheres; we approximated these by rational functions for computational expedience. Lubrication forces between adjacent DNA beads were excluded because lubrication forces are not defined if beads overlap. However, HI between adjacent DNA beads in \((M^+)^{-1}\) cannot be excluded, as such exclusions lead to a loss of positive definiteness in \(M^+\). A correction to this matrix due to possible overlap of adjacent DNA beads was applied, using recently developed formulae \((35)\). The matrix \((M^+)^{-1}\) was formed every 50 time steps, which involves using Ewald summation for \(M^+\) \((36)\), whereas \(R_{\text{lab}}\) was formed every time step. We note that a model for steric interactions is not necessary because lubrication forces prevent particles from overlapping (except for adjacent DNA beads just mentioned). SD simulations used Fixman’s midpoint method for numerical integration \((37,38)\), which handles the nondivergence free nature of the SD resistance matrix.

All simulations were performed using nondimensional units, with a nondimensional time step size of \(10^{-2}\), or 1.64 ps. Particle positions were recorded for analysis every 1000 time steps, i.e., every 0.1 units of time. One unit of time corresponds to the transition between short-time diffusion to long-time diffusion (the characteristic time interval that a particle in a monodisperse suspension needs to escape its cage of immediate neighbors). Table 1 shows the simulation lengths that were used in this study.

**RESULTS**

**DNA internal motion is significant**

To measure 3D diffusion rates, Brownian dynamics simulations without HI were performed on the LacI-DNA system of Fig. 1. In these simulations, LacI was modeled as electrically neutral to measure diffusion without any kind of binding to DNA. Fig. 2 shows the mean square displacements (MSD) for LacI and DNA beads as a function of the time interval considered. In this and the following figures, MSD is measured in units of \(a^2\), where \(a = 1.59 \) nm, the radius of a DNA bead. In this article, diffusion constants are reported in units of \(D_0\), where \(D_0\) is the infinite-dilution diffusion constant for a particle of the same size as a DNA bead, i.e., as follows:

\[
D_0 = \frac{k_B T}{6\pi \eta a} = 154 \ \mu m^2/s,
\]

where \(k_B T\) is the Boltzmann constant times temperature and \(\eta\) is the viscosity of water, all under physiological conditions. We also use the convention that time is measured in units of \(a^2/D_0\), or \(\sim 16.4\) ns.

The diffusion constants of LacI and DNA beads were computed from fitting the data in Fig. 2, ignoring the anomalous diffusion regime at small time intervals, giving 0.0264 and 0.0109 for LacI and DNA, respectively. We note that in dimensional units, the LacI diffusion constant is \(4.07 \mu m^2/s\), nearly the 3\(\times\) measured in vivo with fluorescence correlation microscopy of LacI-Venus without its DNA binding domain \((6)\). An important result from this data is that the diffusion constant for DNA beads is significant compared to the diffusion constant for LacI. DNA is not nearly motionless, as often assumed, compared to the motion of large proteins such as LacI.

We also performed simulations for a much smaller LacI-DNA system, with 256 DNA beads, to check the effect of system size on the results. The resulting diffusion constants are shown in Table 2 compared with the results for the large system already discussed. The diffusion constant for DNA beads for the small system is somewhat larger but comparable to that for the large system. In particular, this shows

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**TABLE 1 Simulation Lengths for Large, 6912 Bead DNA, and Small, 256 Bead DNA, Models**

<table>
<thead>
<tr>
<th>Simulation Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>6912 Bead DNA model (FD only)</td>
<td>11,000.0</td>
</tr>
<tr>
<td>LacI 3D diffusion</td>
<td>11,000.0</td>
</tr>
<tr>
<td>LacI 1D sliding (nonspecifically bound)</td>
<td>12,532.2</td>
</tr>
<tr>
<td>256 Bead DNA model (FD and SD)</td>
<td>9,319.5</td>
</tr>
</tbody>
</table>

Time is measured in units of \(a^2/D_0\) \(\approx 16.4\) ns. For each unit of time, \(10^4\) time steps were used.

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that for large DNA models, DNA internal motion is not significantly slowed down. In contrast to polymers in dilute solutions, where longer polymers experience a slowdown in motion compared to short polymers, in our DNA models, strong internal forces due to compaction and coiling appear to result in significant DNA internal motions even in large systems.

To illustrate the crowded environment of LacI in the nucleoid, Fig. 3 shows a scatter plot of the closest DNA bead to LacI over time. At time 0, LacI is closest to bead 44, but LacI also moves closer to many other DNA beads in a short period of time. Referring to Fig. 1D, LacI is surrounded by several DNA segments. At a later time (for example, near time 4000), LacI appears to come into contact with six different segments of DNA. Near time 2500, LacI appears to transition from one cage of DNA segments to another cage of DNA segments. Fig. 4 shows this behavior in the trajectory of LacI near the transition. This behavior is similar to that of gated diffusion in protein-ligand binding, where the protein must be flexible for the ligand to migrate to its binding site (39).

We conclude that DNA constantly undergoes significant motions, and this mobility of DNA is required for LacI to diffuse around DNA strands. We verified that the motion of the center of mass of the DNA is insignificant in our simulation results. See Movie S1 for an animation of part of the large LacI-DNA simulation.

**Hydrodynamic interactions reduce LacI motion but play a lesser role in DNA internal motion**

In the above simulations, HI was neglected. To more accurately determine the LacI and DNA bead diffusion constants, simulations were also performed using the SD method (23). SD models both short- and long-range HI. The former (also called lubrication forces) is particularly important for accurately modeling high volume fraction systems such as the nucleoid. SD simulations can closely reproduce diffusion constants of proteins in vivo, suggesting that short-range HI are important for accurate modeling of such systems (28). We also previously used SD simulations to study the effect of cellular confinement on macromolecular diffusion (40). We note that we found Brownian dynamics simulations using the Rotne-Prager-Yamakawa diffusion tensor (modeling long-range HI only) to be inappropriate in certain coarse models with crowded conditions, as found previously by other authors (41), as these methods greatly overestimate diffusion constants in our systems,
perhaps due to excessive collective motions of DNA strands.

We performed SD simulations using the smaller LacI-DNA system, with 256 DNA beads, due to the high computational cost of the SD method. These results can be compared to the results shown above for BD simulations without HI, which we denote by FD (free-draining approximation). Fig. 5 shows the MSFs for the small LacI-DNA system using the FD approximation and SD, with results for LacI and for DNA beads given separately. Computed diffusion constants from fitting this data are shown in Table 3.

When HI is considered using the SD method, we observe two major effects. First, the LacI diffusion constant is reduced such that it is comparable to the diffusion constant for DNA beads. We note that in dimensional units, the LacI diffusion constant from SD simulations is $1.89 \, \text{m}^2/\text{s}$, once again close to the $3 \, \text{m}^2/\text{s}$ measured in vivo (6). (For reference, the diffusion constant of glyceraldehyde-3-phosphate dehydrogenase, a protein of similar size to LacI, was measured with SD simulations to be $\sim 10 \, \text{m}^2/\text{s}$ in a model cytosol (28).) The reduction in the diffusion constant in the presence of HI is because particles can feel and interact with other particles without having to collide with them. The second major effect is that the diffusion constant for DNA beads is essentially unchanged in FD and SD simulations, showing that HI plays little role in DNA diffusivity. The strong internal forces due to compacted DNA appear to dominate the hydrodynamic forces.

A movie of the SD simulation trajectory (Movie S2) shows that the DNA chain undergoes various motions, but lengthwise, reptation-like motions (42) along the chain stand out. These types of motions often encounter fewer steric hindrances.

**DNA internal motions effectively increase LacI diffusion when LacI is nonspecifically bound**

One-dimensional diffusion of nonspecifically bound proteins along DNA is believed to primarily consist of sliding along DNA (43, 44), and this sliding is coupled with rotation along the helical track of DNA (14, 45–47). Our model of DNA is too coarse to be able to reproduce this rotation-coupled sliding. However, 1D diffusion can be studied as...
follows. We first note that the experimentally measured LacI 1D diffusion constant of 0.046 μm²/s is 65 times smaller than the LacI 3D diffusion constant (6), although the former is measured in vitro and the latter in vivo. Other studies found that 1D diffusion constants are distributed over a wide range, $2.3 \times 10^{-4}$ to $1.3 \times 10^{-1} \text{μm}^2/\text{s}$ (48,49), with a mean much lower than 3D diffusion. Because the diffusion constant for DNA internal motions (comparable to LacI 3D diffusion) is much greater than the LacI 1D diffusion constant, if observed over short periods of time, sliding LacI appears fixed at a single position on the DNA, whereas its environment changes substantially due to DNA motion. Thus, when LacI dissociates after a sliding event, it has the opportunity to reassociate at a location on a potentially unvisited segment of the DNA. LacI has effectively been transported by DNA internal motions during its 1D sliding process, effectively increasing its diffusion through the nucleoid.

To explore this idea quantitatively, we performed simulations with the FD approximation on the large LacI-DNA system with LacI restrained to a specific DNA bead by a stretch potential, i.e., we assume LacI is nonspecifically bound but does not slide appreciably along DNA during the simulation. The resulting diffusion constant of DNA beads is measured to be 0.0111, essentially unchanged from the result from unrestrained simulations. The diffusion constant of LacI is measured to be 0.0100, essentially the same as that for DNA beads. Therefore the diffusion constant of LacI is controlled by the diffusion constant of DNA beads.

To understand how DNA internal motions can help accelerate protein target search when LacI is nonspecifically bound, we plot in Fig. 6 the cumulative number of contacts that LacI makes with DNA beads in the case of LacI nonspecifically bound to a fixed site on the DNA (labeled “1D diffusion”) and the case of free LacI (labeled “3D diffusion”). At each simulation snapshot, every 0.1 units of time, LacI is said to be in contact with a DNA bead (or one of its periodic images) if that bead is the bead closest to LacI. Given the crowded DNA environment, the closest DNA bead is not always the bead to which LacI is nonspecifically bound. Fig. 6 shows that even in the case of LacI bound to a fixed site (emulating 1D diffusion), LacI comes into contact with a large number of DNA beads. This suggests that internal DNA motions can move DNA segments long distances in relation to other segments.

### The LacI diffusion constant for 1D hopping is large compared to experimentally measured diffusion constants for 1D sliding

The lac operator binding region of LacI is oriented on one side of the protein. Nonspecifically bound LacI may dissociate, diffuse, and then reassociate to the same segment of DNA rather than another segment because of the orientation of the binding region. In contrast to sliding, this type of 1D diffusion along DNA when LacI is not nonspecifically bound is called “hopping”. To try to quantify the LacI 1D diffusion constant for hopping in a packed nucleoid, we performed simulations with electrostatically charged LacI.

In these simulations, a charge is placed on the surface of LacI, corresponding to its binding domain, and the location of this charge at each time step depends on the positions of nearby DNA beads on the given DNA strand along which LacI is hopping. More precisely, at the beginning of a simulation, the DNA bead that is closest to LacI is called the “facing” bead. At the beginning of each time step, a positive charge is placed on the surface of the LacI sphere at the position that is closest to the center of the facing bead. A Debye-Hückel force is computed between the positive charge and all DNA beads (or their nearest periodic images), which are negatively charged. The force on the LacI sphere due to any DNA bead (and vice-versa) is computed using the component of the Debye-Hückel force along the center of the DNA bead and the LacI sphere. These forces restrain LacI along the segment of the DNA with the facing bead. The value of the positive charge is fixed and chosen such that the typical Debye-Hückel energy is approximately the nonspecific binding energy, $10$–$15 \ k_BT$ (50). At the next time step, the facing bead is chosen to be the bead that is closest to the new position of LacI, among the previous
facing bead and the two beads adjacent to it (one ahead and one behind) along the DNA chain.

We performed simulations with the FD approximation and with SD, both using the small DNA model. In simulations, LacI is always very close to the DNA segment along which it was designed to slide, i.e., LacI does not diffuse away from the segment, which would be possible if the binding energy is too small, nor does LacI come so close to a DNA bead such that they overlap in SD simulations.

The linear displacement of LacI along the DNA chain was computed for FD and SD simulations. Linear displacement, measured every 0.1 units of time, is the component of the displacement along the vector joining the facing bead and the bead adjacent to the facing bead that is closest to LacI. The 1D diffusion constant for the LacI linear displacements is 0.0981 (or 15.1 \( \mu \text{m}^2/\text{s} \)) for simulations with the FD approximation, and 0.0189 (or 2.9 \( \mu \text{m}^2/\text{s} \)) for SD simulations. Comparing the results shown in Table 3 for 3D diffusion, and dividing 1D diffusion constants by three to convert to 3D diffusion constants, we find that 1D hopping and 3D diffusion have comparable diffusion constants in simulations with the FD approximation. When accounting for HI using SD simulations, however, a slowdown in 1D hopping diffusion is observed compared to 3D diffusion. In both cases, however, 1D diffusion due to hopping is still rapid, much more rapid than experimentally measured values of 1D diffusion due to sliding (6,48,49). (To the best of our knowledge, there are no experimentally measured values of 1D diffusion due to hopping.)

When LacI is hopping along DNA, we again can observe that the LacI motion is confined within a cage of DNA segments, and then may move into another cage in its diffusion process. Fig. 7 shows the index of the closest DNA bead to LacI over time when LacI is hopping on DNA. The results are for the small system simulated using the FD approximation. The red highlighted points indicate the facing bead on the DNA segment along which the LacI is hopping. It can be observed, for example, that the LacI is caged within at least six DNA segments at time 3000, and enters and exits this cage at times around 2000 and 4000, respectively. Comparing this figure with Fig. 3 for 3D diffusion without nonspecific interactions of the LacI with DNA, gated diffusion is evident in both cases.

**DISCUSSION**

Target search by facilitated diffusion of proteins consists of alternating stages of 3D diffusion between strands of DNA and 1D diffusion along these strands (51,52). The combination of 1D and 3D search can be faster than 1D and 3D search alone. The stages of 1D diffusion help accelerate 3D search by examining multiple sites along DNA (rather than a single site) once the protein is nonspecifically bound. The stages of 3D diffusion help accelerate 1D search by hopping between DNA segments to cut short revisiting the same sites typical of 1D diffusion search. The optimal search time is given when the time spent in 3D diffusion is approximately equal to the time spent in 1D diffusion (52). Unfortunately, it has been found that 1D sliding is very slow and that proteins spend a large majority of their time in 1D search compared to 3D search (6,48,49).

The optimal search time, however, is based on the assumption that the locations along the DNA where a protein nonspecifically rebinds after a 3D search stage to begin a 1D search stage are independent. This is only true in the limit of long intervals of 3D search. Assuming immobile DNA, it is likely that the rebinding points are physically nearby, and thus 1D search is likely to reinitiate at points where 1D search was performed earlier (15). To complicate matters, the conformation of the DNA may play a role: for a straight DNA chain, 3D diffusion is from one site to a sequentially nearby site on the chain; for DNA in a coiled conformation, 3D diffusion more easily reaches a sequentially far away site on the chain. This was systematically studied by Hu et al. (15); the conformations we use correspond to a globule in their work. However, it has also been found that when DNA is packed with sequentially far-away segments being physically near each other, DNA conformation has little effect (21), with the additional assumption that the DNA is straight on the scale of the sliding length.

Previous studies assumed that DNA strands are immobile. If DNA strands are mobile, then during the time that a protein is performing a 1D search, the environment around the strand of DNA to which the protein is nonspecifically bound may change, i.e., strands of DNA that were originally far from the protein may now be nearby. When the protein unbinds, it may rebind to these strands of DNA. The accessible
rebinding site for a finite 3D search time can be imagined as being within a larger radius (equivalently, faster 3D diffusion) than when DNA is immobile. For proteins with two binding domains, DNA internal motions help a protein associated via one domain to quickly find targets for its second binding domain. In these ways, DNA internal motions may accelerate protein target search. These important effects of DNA internal motions were proposed by Berg (22) and studied analytically for wormlike chains, using the term “segmental diffusion” to describe DNA spatial fluctuations. See Lomholt et al. (16) for a more recent treatment.

The results of our study are significant because they are a first step toward quantifying DNA internal motions. We find that DNA internal motions are substantial and can dictate the motions of large transcription factors such as LacI when they are caged or when they are nonspecifically bound. Thus, DNA internal motions compensate for slow 1D sliding.

Our computer studies also give a picture of how large proteins diffuse in a densely packed nucleoid. These proteins are mostly trapped in cages consisting of strands of DNA, but move between cages through a process of gated diffusion. Considering the overall motion of the protein, including its motion dictated by the motion of the cages, the 3D diffusion constant of LacI in silico closely agrees with experimentally measured values in vivo.

DNA motions may vary quantitatively depending on the conformation of the DNA. The conformation of DNA inside living cells is only beginning to be understood. Chromosome conformation capture measurements of interactions between distinct loci on the DNA give a contact probability for loci separated by a given genomic distance, s. Probabilities for intermediate values of s are fitted to the power-law, $s^{-\gamma}$. In humans, $\gamma$ is close to 1, consistent with a fractal (crumpled) globule (53), where sequentially nearby loci also tend to be nearby in space (see also (54,55)). An equilibrium globule, on the other hand, has a value of $\gamma$ of 1.5. In E. coli, experiments suggest that genomic distance to a central loci is linearly related to spatial distance (56). It has been hypothesized that an elongated fractal globule can also replicate this behavior and thus would be a good model for E. coli DNA conformation (57). Previous models of E. coli DNA in crowded conditions have used a conformation that is a closed self-avoiding walk (58).

In our study, we generated a DNA configuration by beginning with DNA beads arranged along a closed space-filling curve where the shortest straight segments are twice the persistence length (Fig. 8 A). Thus the DNA is in a relaxed state, but occupies a large volume. The DNA is placed within a periodic simulation box that is then slowly reduced in dimensions until a physiological DNA volume fraction is reached. This naturally compacts and coils the DNA depending on the stretching and rigidity parameters of the DNA model. The resulting configuration used to initialize our simulations (Fig. 8 B) has a territorial structure like a fractal globule. However, because the system is small and lacks sufficiently many levels of self-similarity, its contact probabilities are more consistent with those of an equilibrium globule (Fig. 8 D), with scaling exponent $\gamma \approx 1.5$. We note that the territorial structure of fractal globules is long lived; we observe that the territorial structure mostly persists even at time 10,000 (Fig. 8 C). Our choice of a closed DNA chain (i.e., a loop) helps the DNA chain remain entangled (57,59).

In our idealized model of DNA and its simulation, several effects were neglected. We did not consider DNA conformation changes as they do during the cell cycle (60). Further, our idealized model did not consider the role of nucleoid-associated proteins that bend DNA or their effect as blockers on DNA (see, for e.g., (17–20) for such a treatment).

The volume fraction or degree of confinement is also a critical parameter in the dynamics of DNA. Kang et al. (61) found that above a critical volume fraction DNA may exhibit glassy dynamics, which is associated with very

**FIGURE 8**  (A) Shown here are 6912 DNA beads arranged along a closed space-filling curve where straight segments are not shorter than twice the persistence length. Red to white to blue coloration varies along the curve. This configuration is placed in a periodic simulation box that is slowly reduced in volume until the desired DNA volume fraction is reached. (B) Shown here is the initial DNA configuration for simulation after the simulation box volume has been reduced. Colors suggest a territorial structure. (C) Given here is the DNA configuration at time 10,000 of simulation with 3D LacI diffusion and mobile DNA. (D) Shown here is the DNA bead contact frequency (proportional to probability in our case) for the initial configuration. Two DNA beads are considered as being in contact if they are within four DNA bead radii of each other. The solid line corresponds to a power-law scaling exponent of $\gamma = 1.5$. The value for $\gamma$ for the configuration at time 10,000 is nearly identical. To see this figure in color, go online.
slow equilibration of fractal globules toward equilibrium globules. It has thus been critical in our work to correctly estimate the volume fraction of DNA in a typical *E. coli* nucleoid. However, such volume fractions change in the lifecycle of a cell, and given that there could be a large variation in volume fraction for different organisms, it would be valuable in the future to study DNA diffusion effects over a range of parameters, as well as alternative DNA structural organizations using larger models. However, our main conclusion stands that DNA internal motions are significant and likely play a role in accelerating overall protein motion within the nucleoid as the protein searches for specific sequence regions of DNA.

**SUPPORTING MATERIAL**

Two movies are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(17)30499-X.

**AUTHOR CONTRIBUTIONS**

E.C. and J.S. designed research. E.C. performed research. E.C. and J.S. analyzed data. E.C. and J.S. wrote the article.

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