THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Mechanism of Facilitated Diffusion during a DNA Search in Crowded Environments

Dana Krepel,[†] David Gomez,[‡] Stefan Klumpp,[‡,§] and Yaakov Levy*[,†]

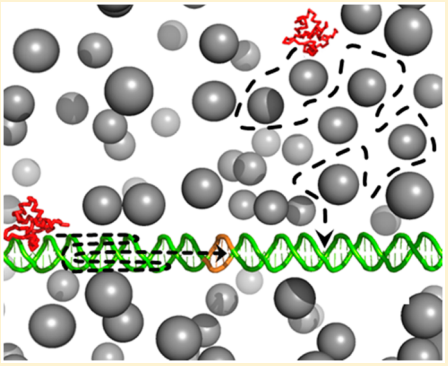
[†]Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

[‡]Max Planck Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam, Germany

[§]Institute for Nonlinear Dynamics, Georg-August University Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Supporting Information

ABSTRACT: The key feature explaining the rapid recognition of a DNA target site by its protein lies in the combination of one- and three-dimensional (1D and 3D) diffusion, which allows efficient scanning of the many alternative sites. This facilitated diffusion mechanism is expected to be affected by cellular conditions, particularly crowding, given that up to 40% of the total cellular volume may by occupied by macromolecules. Using coarse-grained molecular dynamics and Monte Carlo simulations, we show that the crowding particles can enhance facilitated diffusion and accelerate search kinetics. This effect originates from a trade-off between 3D and 1D diffusion. The 3D diffusion coefficient is lower under crowded conditions, but it has little influence because the excluded volume effect of molecular crowding restricts its use. Largely prevented from using 3D diffusion, the searching protein dramatically increases its use of the hopping search mode, which results in a higher linear diffusion coefficient. The coefficient of linear diffusion also increases under crowded conditions as a result of increased collisions between the crowding particles and the searching protein. Overall, less 3D diffusion coupled with an increase in the use of the hopping and speed of 1D diffusion results in faster search kinetics under crowded conditions. Our study shows that the search kinetics and mechanism are modulated not only by the crowding occupancy but also by the properties of the crowding particles and the salt concentration.



INTRODUCTION

The phenomenon of specific molecular recognition between two biomolecules lies at the heart of many biological processes. In the case of interactions between a DNA-binding protein (DBP) and its specific target DNA sequence, recognition requires that the DBP locates its target site among many alternative sites that have a sequence similar to that of the specific target site. Clearly, a failure to rapidly find the target site may result in cellular malfunction because these binding events are often part of a cascade of various essential events in which timing is critical.

The experimentally observed speed of the DNA target search conducted by DBPs can be resolved only by considering a search mechanism involving facilitated diffusion in which the DBPs find their target sites on the DNA through a combination of one- and three- dimensional (1D and 3D) diffusion.[1−6] One-dimensional diffusion includes relatively short bidirectional random walks during which the DBP performs a coupled rotation−translation motion as it moves along the linear contour of the DNA (sliding) combined with short-range jumps between neighboring DNA segments (hopping). Three-dimensional diffusion events involve dissociation from the DNA to the bulk followed by reassociation, thus allowing the DBP to visit DNA regions that are sequentially distant. The physics and biochemistry of the facilitated diffusion mechanism have been investigated from both the theoretical and experimental perspectives, including through complex kinetic models, simulation tools (both at the coarse-grained and atomistic levels),[5,7−14] and in vitro biochemical measurements at the bulk[15−19] and single-molecule levels.[20,21]

The in vivo cellular environment, which is influenced by DNA packing, the positions of nucleosomes, high concentrations of metabolites and macromolecules, and the involvement of other proteins in many regulatory processes, is complex and may directly and indirectly affect the search kinetics and mechanisms. This cellular complexity may dynamically affect the biophysics of facilitated diffusion in various ways. Although some aspects of the DNA search in vivo have been investigated experimentally[22] and some from the theoretical perspective,[23−25] much remains to be understood, particularly in the context of the crowded cellular environment.[26]

The search dynamics, which is governed by nonspecific protein−DNA interactions, is dominated by electrostatic forces and is therefore strongly dependent on the salt concentration, as this influences partitioning between the 1D and 3D search

Received: August 2, 2016
Revised: October 9, 2016
Published: October 10, 2016

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

modes of the facilitated diffusion process. At a low ionic strength, the electrostatic interactions between the DBP and DNA are stronger, and the protein mostly diffuses along the DNA major groove using a relatively short bidirectional 1D random walk. As the salt concentration increases, the protein may move away from the DNA and diffuse into the 3D bulk or remain in the vicinity of the DNA and undertake a linear search via hopping, which is accompanied by a higher diffusion coefficient and enhanced DNA scanning.[5] However, such a search mechanism takes place in a crowded cellular environment in which additional macromolecules may be adsorbed onto the DNA and impose a physical constraint on the search process,[22,27−31] as shown in Figure 1. Given that the

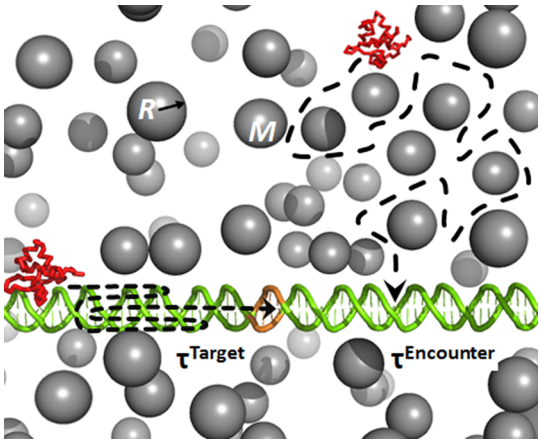


Figure 1. Schematic of DNA search by a protein in the presence of crowding particles. The DNA-binding protein and the spherical crowding particles are shown in red and gray, respectively. The double-stranded DNA is shown in green, and the target site, in orange. The crowding condition is characterized by the volume fraction of the crowding particle, φ, which is affected by the number of particles, N, and their radius, R. The mass of the particle, M, may also affect the overall crowding effect. Crowding may affect the characteristic time needed to localize the target site, τ^{Target} (dashed black line) and the time needed to reach the DNA when starting from a position in the bulk, $\tau^{\text{Encounter}}$ (dashed black line). The crowding condition shown corresponds to a fractional volume of φ = 50%.

macromolecules occupy 10−40% of the total cellular volume (which corresponds to a concentration of ∼100−300 mg/mL[28,32]), they are expected to have a non-negligible effect on the search kinetics. The macromolecular density of the bacterium *Escherichia coli*, for example, slows down 3D diffusion by an order of magnitude, and about 30% of its DNA is associated with proteins, which are mostly bound with no sequence specificity. Macromolecular crowding can, in principle, affect the kinetics of the DNA search in various ways. For example, crowding involving large molecules can exclude some of the 3D volume from the purview of the smaller searching protein in what is known as the exclusion effect (also called depletion forces). Macromolecular crowding can also act as obstacles and block the 1D dynamics of the searching protein along the DNA, thereby truncating its sliding track into short fragments.[33−35] As many DBPs utilize a mixture of the 1D and 3D search mechanisms to facilitate the localization of their DNA target,[1] it is interesting to explore the effect of molecular crowding on the efficiency of the search and in particular how crowding interacts and potentially interferes with both the 1D and 3D components of the DNA search.

The effect of molecular crowding on the kinetics of protein−DNA interactions has shown that facilitated diffusion is affected by the cellular conditions.[36,37] In particular, a recent study suggested that a crowded cellular environment could enhance the mechanism of the DNA search. Apparently, by creating low-viscosity microenvironments around the enzyme and DNA, crowding increases the likelihood that the enzyme will successfully translocate between its respective target sites without dissociating into the bulk solution and also increases the average translocation distance. Furthermore, the protein can traverse a larger linear distance on the DNA chain in the presence of crowding.[36] Although the effect of crowding on protein−DNA recognition cannot be generalized because of the scarcity of data, it should be mentioned that crowding seems to have diverse effects on protein stability[31,38,39] and protein−protein recognition.[40−44] Theoretical studies have suggested different arguments regarding the role of crowding in DNA search kinetics and efficiency.[45−47] The lack of consistency in these studies reflects that the underlying physical principle governing the effect of crowding on protein−DNA interaction is unclear. Thus, despite considerable theoretical[2,6,23,48−50] and experimental efforts,[51] researchers still lack a detailed description and understanding of the possible effects that macromolecular crowding of 1D and 3D movements exerts on the overall efficiency with which a protein searches DNA.[45]

This study seeks to understand the consequences of cellular crowding on DBP search mechanisms and kinetics. Coarse-grained molecular dynamics (CGMD) simulations of non-specific protein−DNA interactions have recently provided new mechanistic insights into the DNA search performed by various proteins[5,7,52−57] and have captured key aspects of the search features observed experimentally, such as the speed−stability paradox.[58,59] Here, we utilized CGMD as well as Monte Carlo (MC) simulations to elucidate the effect of crowding on the DNA search by DBPs with the aim of furthering the understanding of the complexity of target site recognition in the cell.

MATERIALS AND METHODS

The effect of molecular crowding was studied using off-lattice CGMD and on-lattice MC simulations.

Coarse-Grained Molecular Dynamic Simulation Model. In CGMD simulations, the protein is represented by a single bead per residue placed at the Cα position, and the protein dynamics is governed by its native-state topology.[60] The DNA is modeled by three beads per nucleotide (representing phosphate, sugar, and base) that are positioned in the geometric center of the represented group.

We modeled the double-stranded DNA (ds-DNA) in two different ways. In the first model, given the high persistence length for DNA of about 50 nm, it was represented as a rigid and static molecule. It was modeled in its canonical B form and was centered on and aligned with the Z axis. To incorporate flexibility into the DNA, the second model used a three site per nucleotide (3SPN.1) approach, which was shown to capture the thermodynamic properties of various DNA sequences under different conditions.[61] The crowding macromolecules were represented by uncharged spheres that were initially located at random positions and that occupied a total volume determined by their number (N) and their radius (R). Their velocity depends on their mass (M) (Figure 1). We examine how the search characteristics depend on the fractional volume of the crowders, φ, and the ratio between the radius and mass of the crowders and of the searching protein.[62]

We performed CGMD simulations of human DNA-binding protein Sap-1 (PDB code 1bc8) in the presence of 100 bp ds-DNA of poly GC. Sap-1, a 93 amino acid globular protein with a total of 15 and 6

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

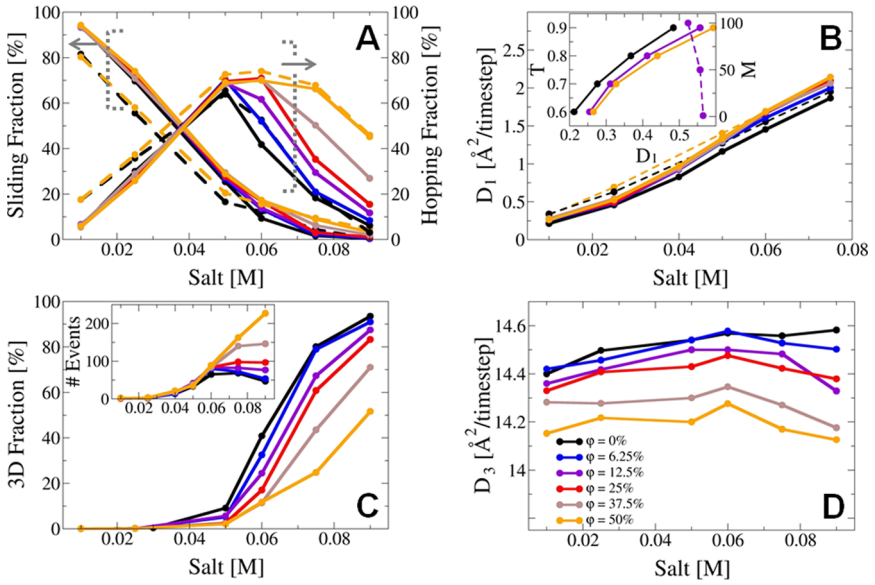


Figure 2. Effect of molecular crowding on the mechanism of DNA search by proteins. (A) The effect of crowding on the use of different types of 1D diffusion (i.e., sliding and hopping, as identified by the dotted brackets and associated arrows). (B) The linear diffusion coefficients (D_1) are shown for different salt concentrations and fractional volumes (φ) of crowding particles. The dotted lines in A and B correspond to protein dynamics along flexible DNA with φ = 0 (dotted black) and 50% (dotted orange), whereas the solid lines in all four subfigures represent protein dynamics along fixed DNA. The inset in B shows the effect of the temperature (solid lines) and mass (dotted line) of the crowder particles on the values of D_1 for different φ conditions. (C) The propensity of the protein to search using 3D diffusion under different salt concentrations and for different fractional volumes and (inset) the number of dissociations from 1D to 3D. (D) The D_3 diffusion coefficient in the bulk for different salt concentrations and fractional volumes. Errors are typically 5−10%.

positively and negatively charged residues, respectively, uses a winged-helix DNA-binding domain to activate transcription. The protein was simulated by a native topology-based model that excluded nonnative interactions and used the Lennard-Jones potential to represent native contact interactions. Electrostatic interactions acting between all of the charged beads in the system were modeled by the Debye−Hückel potential.[5,63] The salt concentration in the simulations using the Debye−Hückel potential refers to a monovalent salt.

The dynamics of the protein along the DNA was simulated using the Langevin equation[5,64] [$m\, dv(t)/dt = -\gamma v(t) + \xi(t)$, where m and v are the particle mass and velocity, γ is the friction coefficient, and ξ is a random force]. We studied the systems using Langevin dynamics in the overdamped limit. The simulations were performed at a constant temperature below the unfolding temperature of Sap-1 (i.e., $T_{simulation} < T_f$, where T_f is the equilibrium protein folding/unfolding temperature) and were analyzed in terms of sliding and hopping (together termed 1D diffusion) and 3D diffusion. All runs were simulated for 1×10^8 time steps that allow extensive DNA sampling by the protein and transitions among sliding, hopping, and 3D search modes. To differentiate among protein sliding, hopping, and free 3D diffusion, we used the definitions defined in ref 5. One-dimensional diffusion along the DNA was used to calculate the mean-square displacement profiles along the Z axis.[5,53] To address the efficiency of the DNA search, we placed the protein on the ds-DNA at a fixed position near one of its edges at time zero and measured how much time elapsed until the DBP arrived at a target site located in the middle of the 100 bp ds-DNA. The mean arrival time to this predefined target site over 100 simulations is indicated by τ^{Target}, where arriving at the target site was defined as reaching a distance threshold of 17 Å between the recognition helix of the DBP and the phosphate beads of the DNA target site. In a similar manner, we estimated the time required for a DBP that had been placed in the bulk solution at time zero to encounter any nonspecific DNA site (i.e., $\tau^{Encounter}$). τ^{Target} estimates the effect of crowding on 1D diffusion, and $\tau^{Encounter}$ measures the effect of crowding on 3D diffusion.

On-Lattice Monte Carlo Simulations. The crowding particles are modeled on a 3D lattice in a box with volume $V = m_x \times m_y \times m_z l^3 = \Omega l^3$ (where l is the lattice spacing and Ω is the total number of lattice sites), with periodic boundary conditions.[65] In the current study, $m_x = m_z = 15$ and $m_y = 100$. One DNA molecule is modeled as a linear arrangement of lattice sites along the y axis so that the DNA is represented by 100 lattice sites (each DNA lattice sites represents several bp's). The system contains a target site and two types of particles: a DBP searching for its target site (placed in the center of the DNA) and C crowding particles that diffuse freely in non-DNA sites and occupy a volume occupation fraction of $\varphi = C/\Omega$ (Figure 5A). Crowders are not allowed to bind the DNA at any time during simulations.

The dynamics of the crowders and the DBP in the bulk solution is implemented as follows: At each simulation time step (of duration τ), C randomly chosen crowders and the DBP move to one of their six neighboring lattice sites with a probability of 1/6 (reduced in the case of crowders with larger mass, see Supporting Information). Thus, crowders and the DBP diffuse with a 3D diffusion constant of $D_3 = l^2/(6\tau)$. If the chosen lattice site is free, then the move is accepted, but if it is already occupied, then the move is rejected. We thus consider excluded volume, or steric repulsion, to be the only interaction in our lattice implementation. If one of the chosen neighboring lattice sites is part of the DNA, then the move of the DBP onto the DNA is always accepted. Once the DBP is nonspecifically bound, one of two events can take place: it can unbind from the DNA with a rate of $k_{off} = P_{off}\tau^{-1}$, where P_{off} is the DBP unbinding probability, or the DBP can diffuse along the DNA with a 1D diffusion constant of D_1. Here, unless otherwise stated, we consider $D_1 = D_3/10$ because it has been shown experimentally that, in general, $D_3 > D_1$.[20] High values of k_{off} mimic screening at a high salt concentration. The DBP can find the target site through 3D diffusion if it directly hops from the solution to the target or by sliding events along the DNA. After the DBP has found the target, the simulation is stopped. Kinetic data are shown for 2000 binding events.

RESULTS AND DISCUSSION

Mechanism of Facilitation in a DNA Search: Effect of Molecular Crowding on 1D and 3D Diffusion. CGMD simulations can provide insights into the molecular mechanism

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

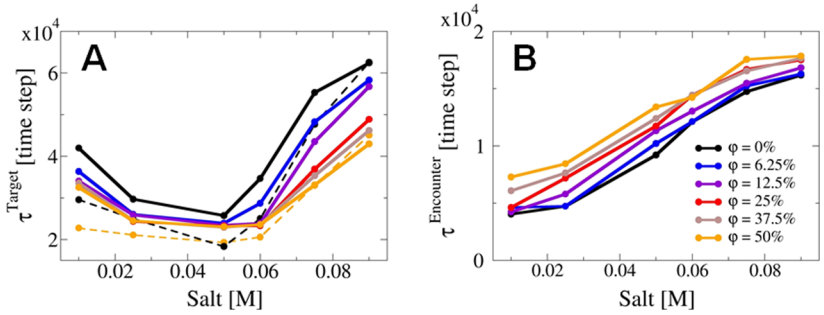


Figure 3. Effect of molecular crowding (modeled as φ, the fractional volume) on the kinetics of the DNA search. (A) The mean time to approach the target site when starting from a selected position on the DNA is indicated by τ^{Target} (Figure 1) at different salt concentrations. The dotted lines correspond to protein dynamics along flexible DNA, with φ = 0 (dotted black) and 50% (dotted orange), whereas the solid lines represent dynamics on fixed DNA. (B) The mean time for the protein to encounter any nonspecific DNA site, $\tau^{Encounter}$, is measured by placing the protein far from the DNA at different salt concentrations. Errors are 25−50% for 0.05/0.06 M. For others, they are typically between 20 and 30%.

underlying enhanced DNA search by a DBP. In particular, the simulations may explain the complex effect of the degree of crowding, salt concentration, and enhanced search kinetics. The effect of crowding on the search dynamics can be elucidated from the search modes (i.e., sliding, hopping, and 3D diffusion) adopted under each condition. Previous studies showed that the search efficiency of a DBP is largely dependent on salt concentration.[5] In this study, we examined how crowding can further affect the search mechanism adopted by the DBP. To address this, we simulated a DBP searching a ds-DNA molecule at salt concentrations ranging from 0.01 to 0.09 M in the presence of crowding macromolecules that had only an entropic effect (i.e., their interactions with the protein or with the DNA were modeled solely by excluded volume). Crowding simulations were performed for various fractional volumes (φ), ranging between 0 and 50%, where the value of φ is a function of the number of crowding particles, N, and their size, R. As control simulations, we studied and compared the search mechanism by the protein in the absence of crowding macromolecules (i.e., φ = 0%).

Figure 2A illustrates that, irrespective of the crowding conditions, the use of the sliding search mode decreases with increasing salt concentration, whereas (at low salt concentrations) that of hopping increases. Interestingly, the utilization of both search modes is unaffected by crowding at relatively low salt concentrations of between 0.01 and 0.05 M even for a high volume fraction of 50%. At higher salt concentrations ([monovalent salt] > 0.05 M), the proportion of sliding adopted by the searching protein is slightly affected by crowding, with more sliding observed at φ = 50% than at φ = 0%. A more profound effect of crowding is observed at higher salt concentrations with respect to the hopping search mode, with a considerably higher proportion of hopping events occurring at φ = 50% than at φ = 0%. The increase in sliding dynamics and especially in hopping dynamics associated with greater crowding occurs at the expense of 3D diffusion, which decreases as crowding increases. For example, upon changing φ from 0 to 50%, the propensity for 3D diffusion decreases from 93 to 52% (Figure 2C). The sharp reduction in productive DBP−DNA dissociation can be attributed to the volume exclusion effect of the crowder molecules, which restrict the ability of the DBP to escape from the DNA and hinder the performance of a 3D excursion in the bulk. When such a 3D event occurs, it is expected to be much shorter for higher φ values because the high viscosity of the crowders will prevent the protein from traversing in solution. Consequently, greater crowding results in greater protein−DNA affinity.[46]

An increase in the number of hopping events under conditions of greater crowding occurs only at moderate and high salt concentrations (Figure 2A), yet faster search kinetics as a consequence of crowding is also observed at a lower salt concentration (Figure 3A). The faster search enabled by crowding at low salt concentrations stems from a different mechanism, which is suggested because in this range of salt concentrations the relative use of the three search modes (i.e., sliding, hopping, and 3D) is very similar. We therefore further analyzed the physical characteristics of the linear diffusion component, which is the most dominant search mode at this salt concentration. Figure 2B shows the 1D diffusion coefficient (D_1) at various fractional volumes under salt concentrations of less than 0.08 M. (Above such salt concentrations, the protein performs mostly 3D diffusion in the bulk.) As can be seen, introducing molecular crowding results in an increase in the D_1 diffusion coefficient at all salt concentrations. Consistent with the pronounced increase in the proportion of hopping with increased crowding (Figure 2A), this increase becomes greater at higher salt concentrations. Introducing flexibility into the DNA molecule (dotted lines in Figure 2A,B) reduced the absolute sliding fraction compared to the values obtained using a rigid DNA molecule and consequently also affected the fraction of hopping. However, DNA flexibility did not alter the effect of crowding or markedly change the shapes of the curves.

To understand the origin of the increase in D_1 at low salt concentrations in the presence of crowding particles, we studied the effect of increasing the temperature or the mass of the crowding molecules on the linear diffusion coefficient. The inset of Figure 2B shows that D_1 increases with temperature irrespective of φ, but at higher values of φ, the increase in D_1 is larger. Nonetheless, we point out that changing the temperature does not affect the fraction of hopping. Increasing the mass of the crowders by a factor of 50 or 100 results in lower D_1 values. The response of D_1 of the DBP to the simulation temperature and to the mass of the crowders suggests that the crowders affect D_1 regardless of a change in the population of the hopping searching mode. Accordingly, at low salt concentrations, the dependence of D_1 on temperature and mass suggests that the crowders constantly collide with the searching protein and consequently D_1 increases. Increasing the temperature or reducing the mass of the crowders increases the frequency of these collisions and further increases D_1. The higher D_1 in the presence of molecular crowding is reminiscent

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

of the higher D_1 reported recently in the context of collisions between a DBP and flexible DNA.[8]

We then examined the effect of molecular crowding on 3D diffusion in the bulk (D_3). Figure 2D shows that D_3 is independent of salt concentration for all values of φ. Opposite to D_1, D_3 decreases as the fractional volume of molecular crowding increases. This suggests that, for large values of φ, the DBP is more confined in 3D and that its 3D diffusion is slower in this viscous environment. Crowding causes D_3 to decrease by up to 2% (Figure 2D) whereas it causes D_1 to increase by up to 15% (Figure 2B). D_3 linearly depends on φ, indicating that it follows the Einstein relation where the viscosity, η, depends linearly on the fraction volume, φ (Figure S1).

Effects of Crowding Fractional Volume on the Kinetics of Finding the DNA Target Site. Molecular crowding may affect the kinetics of the DNA search by affecting the nature of 1D and 3D diffusion. The size of the effect is expected to depend on the fractional volume of the crowders (i.e., on φ) and on their properties, such as their dimension, mass, or affinity for DNA molecules or proteins. The crowders are expected to affect both 1D and 3D diffusion. We therefore designed simulations to quantify these two effects independently. To estimate the effect of crowding on 1D diffusion, we measured τ^{Target}. The effect of crowding on 3D diffusion was estimated by $\tau^{\text{Encounter}}$. The values of τ^{Target} and $\tau^{\text{Encounter}}$ as a function of salt concentration and for different crowding conditions are shown in Figure 3A,B, respectively.

The time needed for a DBP to find its target site regardless of crowding volume fraction strongly depends on the salt concentration, with the fastest search (i.e., smallest τ^{Target}) achieved at a moderate salt concentration (~0.05 M, Figure 3A). This salt concentration corresponds to the optimal condition for achieving the most efficient search in terms of the balance between the 1D and 3D search modes. At lower salt concentrations, searching is dominated by 1D diffusion along the DNA (because the relatively weak electrostatic screening that occurs as low salt concentrations promotes tighter nonspecific protein−DNA interactions), whereas at higher salt concentrations, 3D diffusion is much more common. Accordingly, at higher salt concentrations, a high search speed is achieved by a combination of the 1D and 3D search modes. Figure 3A shows that searches proceed faster in more crowded environments than in less crowded ones under all salt conditions, although both the absolute value of τ^{Target} and the size of the difference between the τ^{Target} values for different fractional crowder volumes depend on the salt concentration. For example, whereas crowding at φ = 6.25−50% enhances the search rate by about 30% at low salt concentrations, at high salt concentrations the size of the effect depends on the exact volume fraction. For φ = 6.25%, a mildly enhanced kinetic effect of about 8% is measured, whereas for φ = 50%, the effect is 45%. This suggests that crowding may exert different effects on 1D dynamics compared to 3D dynamics and that these opposite effects do not necessarily cancel out.[46] Nonetheless, we note that the dependency of τ^{Target} on salt becomes weaker with increasing φ, as reflected by the smaller gap between the search kinetics under salt condition extremes and salt condition for optimal search efficiency (Figure S2). This is another manifestation of a more robust search due to crowding.[46]

We suggest that at moderate and high salt concentrations the faster search kinetics observed with a higher volume fraction of crowding (Figure 3A) is due to 1D diffusion, specifically, hopping, increasing to compensate for 3D dissociation failures. Figure 3B shows that, indeed, at high salt concentrations combined with close crowding the low fraction of 3D is characterized by many short events, whereas in the absence of crowding, 3D diffusion comprises many fewer dissociation events that are very long.

To highlight the influence that crowding exerts on the DBP search kinetics and mechanism, we present (Figure S3) six trajectories of protein dynamics at two crowding volume fractions [φ = 0 (right panels) and 50% (left panels)] and under three representative salt concentrations. These six trajectories illustrate the faster kinetics for binding the target site (lower values of τ^{Target}, indicated by the horizontal red arrow) for φ = 50% compared to φ = 0% and the stronger influence of crowding at higher salt concentrations. The trajectories also illustrate the lower percentage of 3D diffusion at higher crowder volume fractions. At both salt concentrations of 0.06 and 0.09 M, dissociation from the DNA molecule is repressed by the crowding.

To investigate potential crosstalk between molecular crowding and the properties of the DNA molecule, we also studied the effect of crowding on search kinetics using a model that captures DNA flexibility. We found that, in the absence of crowding, τ^{Target} is shorter and the search speed increases by about 30% when the DNA molecule is flexible rather than rigid, although the overall nature of the search remains unchanged (Figure 3A, dotted lines). The small effect of DNA flexibility on partitioning among sliding/hopping/3D diffusion seen in our study is in accordance with some earlier reports.[7,66] The effect of crowding on the time required to locate the target site is similar for rigid and flexible DNA.

Crowding also strongly affects $\tau^{\text{Encounter}}$. In the absence of crowders, $\tau^{\text{Encounter}}$ increases with increasing salt concentration (Figure 3B). The slowing of the time required to achieve DBP−DNA association that occurs as salt concentration increases originates from weaker DBP−DNA attraction and stronger screening. The effect of crowding on $\tau^{\text{Encounter}}$ is simpler than its effect on τ^{Target}. Figure 3B shows that, regardless of salt concentration, the average time needed to achieve nonspecific protein−DNA association increases as the volume fraction increases. $\tau^{\text{Encounter}}$ is particularly long for high values of φ as a result of the strong caging effect on the DBP, which restricts its 3D diffusion. The longer $\tau^{\text{Encounter}}$ at high volume fractions due to confinement by the crowders is reflected in the lower D_3 value as φ increases (Figure 2D).

Finally, we measured τ^{Target} after initially placing the DBP far from the DNA. The results (Figure S4) show that the time needed to find the target site can be governed by the effect of crowding on both 1D and 3D diffusion. Figure S4 shows that the total time resembles that of τ^{Target} when placing the protein on the DNA, which suggests that the DNA search under crowded conditions is dominated by the time scale of linear diffusion rather than 3D diffusion.

Effect of the Mass of the Crowders on the Kinetics of the Facilitated Diffusion. To understand better the effect of crowding, we investigated how the mass of the crowders may influence their ability to modulate the kinetics of searching the DNA. Up to this point, the presented results were obtained using crowder molecules with a constant small mass of M = 1. The mass of each amino acid bead of the DBP was also 1, such that the DBP was 93 times heavier than a crowding molecule. In order to examine mass effects, we repeated the calculations for larger crowder particles with mass of either M = 50 or M = 100.

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

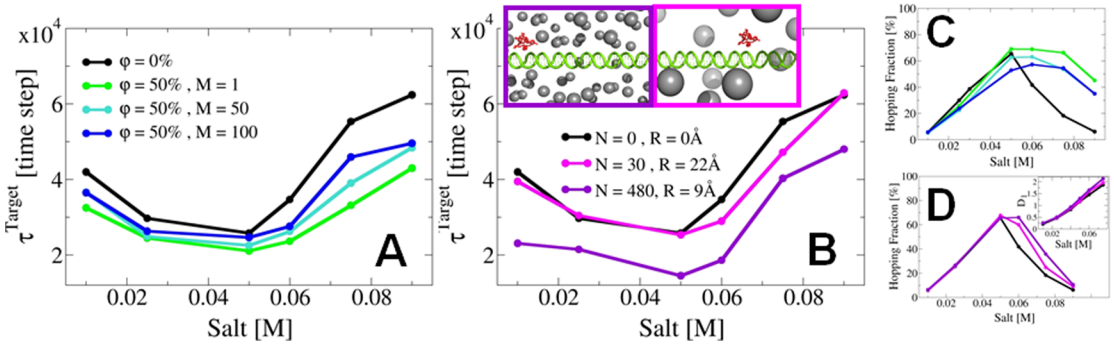


Figure 4. Effect of the mass and dimension of the crowding particle on the DNA search. (A) τ^{Target} as a function of salt concentration at a crowder fractional volume (φ) of 0 or 50% for crowding particles of different masses (and R = 9 Å). (B) τ^{Target} adopted by a DBP at a φ of 12.5% at different salt concentrations using crowding particles of R = 0 (no crowding particles), 45, or 18 Å (M = 1). The snapshots illustrate the two conditions of different N and R used to achieve the same fractional volume of 12.5%. The DBP is shown in red, the crowder molecules appear as gray spheres, and the DNA molecule appears in green. (C) Effect of fractional volume on the proportion of hopping utilized by a DBP on DNA for crowding particles with the various masses examined in A. (D) The effect of particle size on the use of hopping as a DNA search mechanism for the same particle sizes as examined in B. The inset shows the diffusion coefficient D_1 as a function of salt concentration during simulated protein scanning of the DNA.

In Figure 4A, we present the effect of mass on τ^{Target} by comparing the value of the latter at φ = 0 and 50% for particles of mass M = 1, 50, or 100 at various salt concentrations. As was discussed above (Figure 3A), the presence of crowding particles facilitates the kinetics of the system by restricting the protein's movement so that it remains closer to the DNA axis, thus enabling it to explore new sites. With increasing mass, τ^{Target} values gradually increase until they approach the kinetics of the dynamics in the absence of crowding (φ = 0%). In addition, Figure 4C shows the use of the hopping search mode in the presence of different crowding masses. Similar to what was seen in Figure 4A, increasing the mass of the particles also diminishes the strong effect of crowding on the proportion of hopping used. The effects of an increase in mass mimic those due to crowding, as was seen in Figure 2A.

A possible explanation of these effects is that introducing slowly moving crowding particles increases the viscosity of the intracellular environment and therefore particle−DBP collisions become less frequent. Heavier crowding particles affect the linear dynamics of the proteins along the DNA differently at different salt concentrations. At low salt concentrations, fewer collisions between higher-mass particles and the DBP (blue line in Figure 4A) result in a smaller linear diffusion coefficient and therefore a slower search (larger τ^{Target}) than that associated with lower-mass particles (green lines in Figure 4A), yet it is faster than in the case of no crowding (black line, Figure 4A). The introduction of higher-mass particles also results is an increase in $\tau^{Encounter}$ (Figure S5), further exemplifying the caging effect of the crowders. At higher salt concentrations, the mass of the crowding particles affects the hopping mode, with the presence of heavier crowding particles causing less hopping and thus slower kinetics for the DBP to find its target site (Figure 4C). We note that changing the mass of the crowders was done in order to examine the potential effect of collisions between the crowders and the searching protein on the search kinetics and that this is related to the effect of crowders on the D_1 value. If the diffusion is in simple liquid that follows the Stokes−Einstein relationship, then the diffusion coefficients of the crowders will be mass-independent, but it is possible that the diffusion in the cell will deviate from this relationship.

Effect of the Size of the Crowders on the Kinetics of Facilitated Diffusion. In addition to the mass of the crowding particles, their dimension (i.e., their radius R) may also affect their overall effect on diffusion along the DNA. Indeed, the effects of molecular crowding on biochemical reactions are known to be dependent on the reactants' size and geometry.[67] Here, the crowding particles are assumed to be spherical, and their dimension can be tuned simply by changing their radii. Specifically, large particles experience entropic attraction forces in the presence of smaller particles, thus smaller particles are expected to have a stronger effect on binding. We note that the effect of the crowder size is solely represented by the excluded volume effects (i.e., the friction coefficient, g, remains unchanged, although by following the Stokes law it linearly increases with the particle radius).

We began by studying the kinetics effect as a function of salt concentration by measuring τ^{Target} with crowding particles that were either smaller or larger (Figure 4B) than the radius of the DBP. A stronger excluded volume effect and thus a more pronounced crowding effect is expected for larger particles, when keeping the concentration (i.e., number of particles per volume unit) of the crowding particles constant. To avoid this trivial scenario, we compared two systems with different particle radii (R = 45 and 18 Å) but the same fractional volume, which we achieved by changing the number of particles, N (Figure 4B). We examined the effect of particle size for φ = 12.5% and compared it to a system with no crowding particles, φ = 0%. Decreasing the size of the crowding particles (while increasing the number of crowding particles in order to keep φ constant) results in a larger effect in which τ^{Target} is smaller than for larger crowding particles. When the crowding particles are smaller, the DBP encountered a larger number of particles, which results in a strong decrease in the values of τ^{Target} compared to the values of the larger particle at all salt concentrations (Figure 4B). As can be seen in Figure 4D, this increase in search efficiency in the presence of crowding particles having smaller dimensions is also accompanied by a greater utilization of hopping at higher salt concentrations than is seen in the presence of larger crowding particles and increases in the values of the D_1 coefficient (inset of Figure 4D).

Monte Carlo Simulations of a DNA Search in the Presence of Molecular Crowding. We supplement the CGMD simulations with dynamic MC simulations, as the latter are valuable in evaluating the effect of modifying some key parameters of the facilitated diffusion mechanism on the kinetics. The moves of the MC simulations are chosen to

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

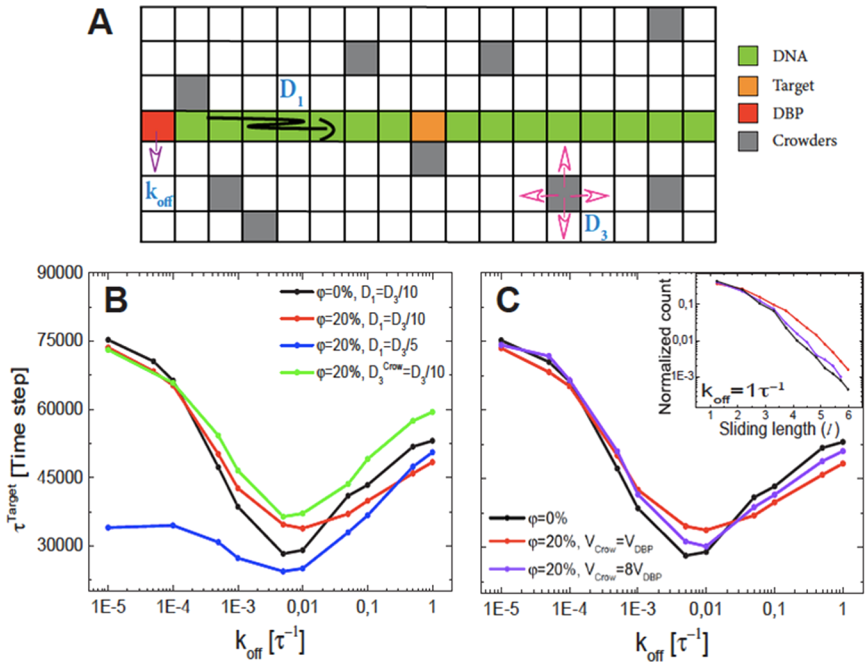


Figure 5. On-lattice MC model for target finding. (A) Schematic view of the model. A DBP, in red, diffuses by hopping either to one of the six nearest-neighbor lattice sites, if it is in the bulk solution, or to one of the two neighboring lattice sites, if it is sliding along the DNA, which is represented in green. Crowders represented in gray diffuse only in the bulk solution and are not allowed to bind the DNA. Molecules have square geometry, and diffusion is allowed only if the destination site is unoccupied. Note that the simulations use a 3D lattice rather than the 2D one depicted schematically here. (B) Average target finding time as a function of k_{off} for different volume occupation fractions φ, 1D diffusion constants D_1, and crowder masses. (C) Average finding time as a function of k_{off} for different crowder sizes. (Inset) Sliding length distributions for different crowder sizes.

closely mimic the physical dynamics (Figure 5A): the diffusion of the protein and of the crowding particles is described by random walks on a lattice;[68] their dynamics is dictated by diffusion coefficients D_1 and D_3. Unbinding from DNA is incorporated as a reduced stepping rate k_{off} from a DNA site to a non-DNA site; the effect of salt is included in the latter parameter. Excluded volume interactions are included by not allowing particles to move to occupied lattice sites. The time scale τ of the MC step is set by the diffusive rate of a particle stepping from one lattice site to a neighboring site, $\tau = l_2/(6D_3)$. In the MC simulations, the protein and the crowding particles move on a lattice, and their dynamics is dictated by the D_1 and D_3 parameters and the salt effect is mimicked via the k_{off} parameter (Figure 5A). Figure 5B shows the average time to find the target site as a function of the unbinding rate k_{off} for different levels of molecular crowding. When no crowders are present, the average finding time exhibits nonmonotonic behavior with its minimum at an optimal value of k_{off}, similar to the CGMD results shown in Figure 3A. As molecular crowding increases, the sliding length increases because of less dissociation from the DNA (Figure S6). This effect is mostly beneficial for large values of k_{off}, but for small values of k_{off} (i.e., low salt concentration), the presence of crowders does not affect the search kinetics.

A fundamental difference between the CGMD and the MC approaches is that in the former the D_1 increases with salt concentration and in the MC D_1 is constant and is decoupled from k_{off}. To examine the effect of D_1 on the search kinetics, we simulated the search under crowded conditions and by utilizing higher values for D_1. Figure 5B shows that lower values of k_{off} paired with higher values of D_1 results in a shorter τ^{Target}; however, large values of k_{off} have no major effect on the search kinetics. The faster search under conditions of low k_{off} and higher D_1 values is similar to the effect reported in Figure 3A, in which crowding produces a shorter τ^{Target} at low salt concentrations.

To implement the different mass of the DBP and the crowding particles in the MC simulations model, we modified the crowder's diffusion constant D_3^{crow}. Figure 5B shows the average finding time as a function of k_{off} for crowding particles that are heavier and diffuse with $D_3^{Crow} = D_3/10$. Increasing the mass of the crowding particles affects the search kinetics only at high values of k_{off}, under which the kinetics is dominated by D_3. When the DBP and the crowders have the same diffusion constant, the excluded volume enhances the target finding. Nevertheless, when crowders diffuse more slowly than the DBP, their effect on target localization is smaller, suggesting that slow crowders affect the 3D diffusion of the DBP more strongly than do crowders with the same diffusivity. This behavior is similar to the results from the CGMD simulations (Figure 4A). We note that, at the limit of very slow crowders, percolation effects can take place, with the result that the DBP would never find its target.

Next, we used the MC model to study crowders that are larger than the DBP. Figure 5C shows the average finding time as a function of k_{off} for two cases in which the volume fractions are the same but the particles have different sizes: a larger number of smaller particles is seen to result in faster kinetics. The origin of this enhanced kinetics is more effective excluded volume effects (inset of Figure 5C). We point out that a similar effect is reported for the CGMD simulations (Figure 4B); however, the effect is not identical, most likely because the CGMD also involves a change in the D_1 value that was not

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

incorporated into the MC simulations, which focused on the effect of the dimensions of the crowding particles.

The MC simulations support the CGMD observations that molecular crowding increases the linear diffusion coefficient D_1, that the presence of lighter crowding particles can speed up the search (by increasing the D_3 value), and that smaller crowding particles may be more effective at enhancing facilitated diffusion.

CONCLUSIONS

Recent studies contributed to the understanding of various facets of the mechanism of DNA recognition by proteins. In addition to the typical complexities of biomolecular recognition, which demand structural and chemical complementarity, the interactions between protein and DNA require an extensive search that is further complicated by conditions in the cellular milieu. The in vivo aspects of protein−DNA recognition are just starting to be addressed, but some studies have surprisingly suggested that the complexity may not significantly exceed the in vitro scenario. Actually, it was indicated that the crowded cellular environment and particularly obstacles on the DNA may not necessarily impede the search kinetics.[33,46,69]

In this article, using CGMD and MC simulations, we demonstrated how molecular crowding influences DBP dynamics as it searches DNA. We have shown that the DBP search efficiency in the presence of crowding may be improved as its dynamics becomes confined to the DNA regions between the crowding particles. This finding may imply that the excluded volume effects of crowding particles can sequester sampling to within certain DNA regions while reducing the possibility of the DBP escaping the DNA. The sequestration effect of crowding may result in a faster search when the target site is located in the accessible region along the DNA. We demonstrate how the search efficiency is altered under varying volume occupancies and show that this effect strongly depends on the salt concentration. We suggest that, at increasing occupancies, crowding has an influence on the partitioning between the searching modes (sliding, hopping, and 3D diffusion) adopted by the DBP, in a similar manner to the effect of decreasing salt conditions.

Although crowding particles may restrict the DNA region that is accessible for searching, their increasing number could alter the nature of hopping events and so influence the value of the D_1 coefficient. This influence becomes considerable at higher salt concentrations, at which the protein performs more hopping at the expense of 3D diffusion. This observation serves as another example of the importance of hopping as a DNA search mode. The enhanced hopping dynamics may also be viewed as an outcome of frequent collisions between the DBP and crowding particles, which increase as the fractional volume of the latter increases. The mass of the particles, their size, and the temperature of the system are also important parameters influencing the characteristics of linear diffusion, in addition to their entropic effect via the excluded volume interactions. At a constant fractional volume, decreasing the mass of the crowding particles, decreasing the dimension of the crowding particles, decreasing their number, or using higher system temperatures increases the number of particle−protein collisions. Consequently, the linear diffusion of the DBP along the DNA is characterized by higher D_1 due to faster sliding or due to heavier use of the hopping searching mode. As a result, crowding effects become more localized and the protein may perform less efficient sampling of the DNA sites and increase its search time. Thus, the synergism among crowding occupancy, the properties of crowding particles, and the salt concentration may facilitate the search and contribute to the ability of proteins to navigate through the complex DNA organization to find their regulatory binding sites.

We thus provide evidence that D_1 can be affected not only by the salt concentration (which changes the hopping propensity) but also by the volume fraction because at low salt concentrations, collisions increase the D_1 of sliding whereas higher salt concentrations increase the hopping propensity. Furthermore, D_3 is also affected by increased crowding because of the effect of confinement, which may restrict 3D diffusion as crowding increases. Thus, this study suggests that crowding may affect the search kinetics not only via excluded volume effects but also by modifying both D_1 and D_3. Thus, it is evident that the volume fraction, φ, is not a sufficient parameter to capture the effect of crowding during the DNA search but rather the molecular properties of the crowding molecules (such as their mass and molecular dimension) may control their overall effect. This is in agreement with a recent study[36] that suggested that a crowded cellular environment could influence the mechanism of DNA damage recognition by an enzyme to the same extent as any property of the enzyme itself or the DNA. We speculate that, in addition to the simple excluded volume effects of inert polymers, the presence of crowding particles with an affinity for either proteins or DNA[36,70] may modify the facilitated diffusion mechanism. Furthermore, it is also expected that crowding may affect the ability of proteins to jump between neighboring DNA segments via the "monkey-bar" mechanism[56] and thus affect the overall effect of crowding conditions on the DNA search kinetics.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b07813.

Detailed kinetic analysis of the effect of the different crowding particles on protein diffusion along DNA from the coarse-grained MD and MC simulations (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: Koby.Levy@weizmann.ac.il. Tel: 972-8-9344587.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Kimmelman Center for Macromolecular Assemblies and the Minerva Foundation, with funding from the Federal German Ministry for Education and Research. Y.L. is The Morton and Gladys Pickman Professional Chair in Structural Biology. D.K. was supported by the Prof. Dov and Ziva Rabinovich memorial fund on Structural Biology.

REFERENCES

(1) Berg, O. G.; Winter, R. B.; von Hippel, P. H. Diffusion-driven mechanisms of protein translocation on nucleic acids. 1. Models and theory. *Biochemistry* **1981**, *20* (24), 6929−6948.

(2) Slutsky, M.; Mirny, L. A. Kinetics of protein-DNA interaction: Facilitated target location in sequence-dependent potential. *Biophys. J.* **2004**, *87* (6), 4021−4035.

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

(3) Halford, S. E. An end to 40 years of mistakes in DNA-protein association kinetics? *Biochem. Soc. Trans.* **2009**, *37*, 343−348.
(4) Gowers, D. M.; Wilson, G. G.; Halford, S. E. Measurement of the contributions of 1D and 3D pathways to the translocation of a protein along DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (44), 15883−15888.
(5) Givaty, O.; Levy, Y. Protein sliding along DNA: Dynamics and structural characterization. *J. Mol. Biol.* **2009**, *385*, 1087−1097.
(6) Sheinman, M.; Benichou, O.; Kafri, Y.; Voituriez, R. Classes of fast and specific search mechanisms for proteins on DNA. *Rep. Prog. Phys.* **2012**, *75* (2), 026601.
(7) Terakawa, T.; Kenzaki, H.; Takada, S. p53 Searches on DNA by Rotation-uncoupled sliding at C-terminal tails and restricted hopping of core domains. *J. Am. Chem. Soc.* **2012**, *134* (35), 14555−14562.
(8) Ando, T.; Skolnick, J. Sliding of proteins non-specifically bound to DNA: Brownian dynamics studies with coarse-grained protein and DNA models. *PLoS Comput. Biol.* **2014**, *10* (12), e1003990.
(9) Echeverria, I.; Papoian, G. A. DNA Exit ramps are revealed in the binding landscapes obtained from simulations in helical coordinates. *PLoS Comput. Biol.* **2015**, *11* (2), e1003980.
(10) Marklund, E. G.; Mahmutovic, A.; Berg, O. G.; Hammar, P.; van der Spoel, D.; Fange, D.; Elf, J. Transcription-factor binding and sliding on DNA studied using micro- and macroscopic models. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (49), 19796−19801.
(11) Zabet, N. R.; Adryan, B. Computational models for large-scale simulations of facilitated diffusion. *Mol. BioSyst.* **2012**, *8* (11), 2815−27.
(12) Chen, C.; Pettitt, B. M. DNA Shape versus Sequence Variations in the Protein Binding Process. *Biophys. J.* **2016**, *110* (3), 534−544.
(13) Bauer, M.; Metzler, R. Generalized facilitated diffusion model for DNA-binding proteins with search and recognition states. *Biophys. J.* **2012**, *102* (10), 2321−2330.
(14) Bauer, M.; Rasmussen, E. S.; Lomholt, M. A.; Metzler, R. Real sequence effects on the search dynamics of transcription factors on DNA. *Sci. Rep.* **2015**, *5*, 10072.
(15) Clore, G. M. Exploring translocation of proteins on DNA by NMR. *J. Biomol. NMR* **2011**, *51*, 209−219.
(16) Iwahara, J.; Zweckstetter, M.; Clore, G. M. NMR structural and kinetic characterization of a homeodomain diffusing and hopping on nonspecific DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (41), 15062−15067.
(17) Schonhoft, J. D.; Stivers, J. T. Timing facilitated site transfer of an enzyme on DNA. *Nat. Chem. Biol.* **2012**, *8* (2), 205−210.
(18) Porecha, R. H.; Stivers, J. T. Uracil DNA glycosylase uses DNA hopping and short-range sliding to trap extrahelical uracils. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (31), 10791−10796.
(19) Pollak, A. J.; Chin, A. T.; Reich, N. O. Distinct facilitated diffusion mechanisms by E. coli type II restriction endonucleases. *Biochemistry* **2014**, *53* (45), 7028−7037.
(20) Elf, J.; Li, G.-W.; Xie, X. S. Probing transcription factor dynamics at the single-molecule level in a living cell. *Science (Washington, DC, U. S.)* **2007**, *316*, 1191−1194.
(21) Tafvizi, A.; Huang, F.; Fersht, A. R.; Mirny, L. A.; van Oijen, A. M. A single-molecule characterization of p53 search on DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (2), 563−568.
(22) Zhou, H. X.; Rivas, G. N.; Minton, A. P. Macromolecular crowding and confinement: Biochemical, biophysical, and potential physiological consequences. *Annu. Rev. Biophys.* **2008**, *37*, 375−397.
(23) Mirny, L.; Slutsky, M. How a protein searches for its site ob DNA: the mechanism of facilitated diffusion. *J. Phys. A: Math. Theor.* **2009**, *42*, 434013.
(24) Bauer, M.; Metzler, R. In vivo facilitated diffusion model. *PLoS One* **2013**, *8* (1), e53956.
(25) Shin, J.; Cherstvy, A. G.; Metzler, R. Self-subdiffusion in solutions of star-shaped crowders: Non-monotonic effects of inter-particle interactions. *New J. Phys.* **2015**, *17*, 113028.
(26) Elcock, A. H. Models of macromolecular crowding effects and the need for quantitative comparisons with experiment. *Curr. Opin. Struct. Biol.* **2010**, *20* (2), 196−206.
(27) Zimmerman, S. B.; Minton, A. P. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22* (1), 27−65.
(28) Ellis, R. J. Macromolecular crowding: Obvious but under-appreciated. *Trends Biochem. Sci.* **2001**, *26* (10), 597−604.
(29) Kim, J. S.; Yethiraj, A. Crowding effects on protein association: Effect of interactions between crowding agents. *J. Phys. Chem. B* **2011**, *115* (2), 347−353.
(30) Muramatsu, N.; Minton, A. P. Tracer diffusion of globular proteins in concentrated protein solutions. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85* (9), 2984−2988.
(31) Cheung, M. S.; Klimov, D.; Thirumalai, D. Molecular crowding enhances native state stability and refolding rates of globular proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (13), 4753−4758.
(32) Minton, A. P. The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J. Biol. Chem.* **2001**, *276* (14), 10577−10580.
(33) Marcovitz, A.; Levy, Y. Obstacles may facilitate and direct DNA search by proteins. *Biophys. J.* **2013**, *104* (9), 2042−2050.
(34) Liu, L.; Luo, K. F. DNA-binding protein searches for its target: Non-monotonic dependence of the search time on the density of roadblocks bound on the DNA chain. *J. Chem. Phys.* **2015**, *142* (12), 125101.
(35) Shvets, A.; Kochugaeva, M.; Kolomeisky, A. B. Role of static and dynamic obstacles in the protein search for targets on DNA. *J. Phys. Chem. B* **2016**, *120* (26), 5802−5809.
(36) Cravens, S. L.; Schonhoft, J. D.; Rowland, M. M.; Rodriguez, A. A.; Anderson, B. G.; Stivers, J. T. Molecular crowding enhances facilitated diffusion of two human DNA glycosylases. *Nucleic Acids Res.* **2015**, *43* (8), 4087−4097.
(37) Akabayov, B.; Akabayov, S. R.; Lee, S. J.; Wagner, G.; Richardson, C. C. Impact of macromolecular crowding on DNA replication. *Nat. Commun.* **2013**, *4*, 1615.
(38) Dhar, A.; Samiotakis, A.; Ebbinghaus, S.; Nienhaus, L.; Homouz, D.; Gruebele, M.; Cheung, M. S. Structure, function, and folding of phosphoglycerate kinase are strongly perturbed by macromolecular crowding. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (41), 17586−17591.
(39) Mittal, S.; Singh, L. R. Denatured state structural property determines protein stabilization by macromolecular crowding: A thermodynamic and structural approach. *PLoS One* **2013**, *8* (11), e78936.
(40) Kim, Y. C.; Best, R. B.; Mittal, J. Macromolecular crowding effects on protein-protein binding affinity and specificity. *J. Chem. Phys.* **2010**, *133* (20), 205101.
(41) Bhattacharya, A.; Kim, Y.; Mittal, J. Protein−protein interactions in a crowded environment. *Biophys. Rev.* **2013**, *5* (2), 99−108.
(42) Miller, C. M; Kim, Y. C.; Mittal, J. Protein composition determines the effect of crowding on the properties of disordered proteins. *Biophys. J.* **2016**, *111*, 28−37.
(43) Zhou, H. X. Influence of crowded cellular environments on protein folding, binding, and oligomerization: Biological consequences and potentials of atomistic modeling. *FEBS Lett.* **2013**, *587* (8), 1053−1061.
(44) Kinjo, A. R.; Takada, S. Effects of macromolecular crowding on protein folding and aggregation studied by density functional theory: Statics. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2002**, *66* (5), 031911.
(45) Li, G.; Berg, O.; Elf, J. Effects of macromolecular crowding and DNA looping on gene regulation kinetics. *Nat. Phys.* **2009**, *5*, 294−297.
(46) Brackley, C.; Cates, M.; Marenduzzo, D. Intracellular facilitated diffusion: Searchers, crowders, and blockers. *Phys. Rev. Lett.* **2013**, *111*, 108101.
(47) Tabaka, M.; Kalwarczyk, T.; Holyst, R. Quantitative influence of macromolecular crowding on gene regulation kinetics. *Nucleic Acids Res.* **2014**, *42* (2), 727−738.
(48) Kolomeisky, A. Physics of protein-DNA interactions: mechanisms of facilitated target search. *Phys. Chem. Chem. Phys.* **2011**, *13*, 2088−2095.

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122

(49) Veksler, A.; Kolomeisky, A. B. Speed-selectivity paradox in the protein search for targets on DNA: Is it real or not? *J. Phys. Chem. B* **2013**, *117* (42), 12695−12701.
(50) Hu, T.; Grosberg, A. Y.; Shklovskii, B. I. How proteins search for their specific sites on DNA: the role of DNA conformation. *Biophys. J.* **2006**, *90* (8), 2731−2744.
(51) Gorman, J.; Plys, A. J.; Visnapuu, M. L.; Alani, E.; Greene, E. C. Visualizing one-dimensional diffusion of eukaryotic DNA repair factors along a chromatin lattice. *Nat. Struct. Mol. Biol.* **2010**, *17* (8), 932−938.
(52) Marcovitz, A.; Levy, Y. Weak frustration regulates sliding and binding kinetics on rugged protein-DNA landscapes. *J. Phys. Chem. B* **2013**, *117* (42), 13005−13014.
(53) Bhattacherjee, A.; Levy, Y. Search by proteins for their DNA target site: 1. The effect of DNA conformation on protein sliding. *Nucleic Acids Res.* **2014**, *42* (20), 12404−12414.
(54) Khazanov, N.; Marcovitz, A.; Levy, Y. Asymmetric DNA-search dynamics by symmetric dimeric proteins. *Biochemistry* **2013**, *52* (32), 5335−5344.
(55) Khazanov, N.; Levy, Y. Sliding of p53 along DNA can be modulated by its oligomeric state and by cross-talks between its constituent domains. *J. Mol. Biol.* **2011**, *408* (2), 335−355.
(56) Vuzman, D.; Azia, A.; Levy, Y. Searching DNA via a "Monkey Bar" mechanism: The significance of disordered tails. *J. Mol. Biol.* **2010**, *396* (3), 674−684.
(57) Vuzman, D.; Polonsky, M.; Levy, Y. Facilitated DNA search by multidomain transcription factors: cross talk via a flexible linker. *Biophys. J.* **2010**, *99* (4), 1202−1211.
(58) Zandarashvili, L.; Vuzman, D.; Esadze, A.; Takayama, Y.; Sahu, D.; Levy, Y.; Iwahara, J. Asymmetrical roles of zinc fingers in dynamic DNA-scanning process by the inducible transcription factor Egr-1. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (26), E1724−E1732.
(59) Zandarashvili, L.; Esadze, A.; Vuzman, D.; Kemme, C. A.; Levy, Y.; Iwahara, J. Balancing between affinity and speed in target DNA search by zinc-finger proteins via modulation of dynamic conformational ensemble. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (37), E5142−E5149.
(60) Noel, J. K.; Whitford, P. C.; Sanbonmatsu, K. Y.; Onuchic, J. N. SMOG@ctbp: Simplified deployment of structure-based models in GROMACS. *Nucleic Acids Res.* **2010**, *38*, W657−W661.
(61) Sambriski, E. J.; Schwartz, D. C.; De Pablo, J. J. A mesoscale model of DNA and its renaturation. *Biophys. J.* **2009**, *96*, 1675−1690.
(62) Kang, H.; Pincus, P. A.; Hyeon, C.; Thirumalai, D. Effects of macromolecular crowding on the collapse of biopolymers. *Phys. Rev. Lett.* **2015**, *114* (6), 068303.
(63) Schlick, T. *Molecular modeling and simulation - An interdisciplinary guide* **2002**, *21*, 298−301.
(64) Mor, A.; Ziv, G.; Levy, Y. Simulations of proteins with inhomogeneous degrees of freedom: The effect of thermostats. *J. Comput. Chem.* **2008**, *29* (12), 1992−1998.
(65) Gomez, D.; Klumpp, S. Biochemical reactions in crowded environments: Revisiting the effects of volume exclusion with simulations. *Front. Phys.* **2015**, *3*, 45.
(66) Mondal, A.; Bhattacherjee, A. Searching target sites on DNA by proteins: Role of DNA dynamics under confinement. *Nucleic Acids Res.* **2015**, *43* (19), 9176−9186.
(67) Minton, A. P. Excluded volume as a determinant of macromolecular structure and reactivity. *Biopolymers* **1981**, *20* (10), 2093−2120.
(68) Gomez, D.; Klumpp, S. Facilitated diffusion in the presence of obstacles on the DNA. *Phys. Chem. Chem. Phys.* **2016**, *18* (16), 11184−11192.
(69) Shvets, A. A.; Kolomeisky, A. B. Crowding on DNA in protein search for targets. *J. Phys. Chem. Lett.* **2016**, *7* (1), 2502−2506.
(70) Benton, L. A.; Smith, A. E.; Young, G. B.; Pielak, G. J. Unexpected effects of macromolecular crowding on protein stability. *Biochemistry* **2012**, *51* (49), 9773−9775.

DOI: 10.1021/acs.jpcb.6b07813
J. Phys. Chem. B 2016, 120, 11113−11122