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Protein diffusion along DNA: on the effect of roadblocks and crowders

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Abstract

Rapid recognition by a protein of its DNA target site is achieved through a 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. Both experimental and theoretical studies showed that crowding particles can enhance facilitated diffusion and accelerate search kinetics. This effect may originate from crowding forcing a trade-off between 3D and 1D diffusion. In this study, using coarse-grained molecular dynamic simulations, we investigate how the molecular properties of the crowders may modulate the effect exerted by crowding on a searcher protein. We show that crowders with an affinity to the DNA are less effective search facilitators than particles whose contribution is solely entropic. Crowders that have affinity to DNA may occupy DNA sites and thereby function as obstacles or roadblocks that slow down the searcher protein, and they may also produce a smaller excluded volume effect and so reduce usage of the hopping searching mode in favor of less-effective 3D diffusion in the bulk. We discuss how strong repulsive interactions between the crowding particles themselves may affect the overall dynamics of the crowders and their excluded volume effect. Our study shows that search kinetics and its mechanism are modulated not only by salt concentration and crowding occupancy, but also by the properties of the crowding particles.

Keywords: DNA search, coarse-grained model, molecular dynamics simulations

(Some figures may appear in colour only in the online journal)

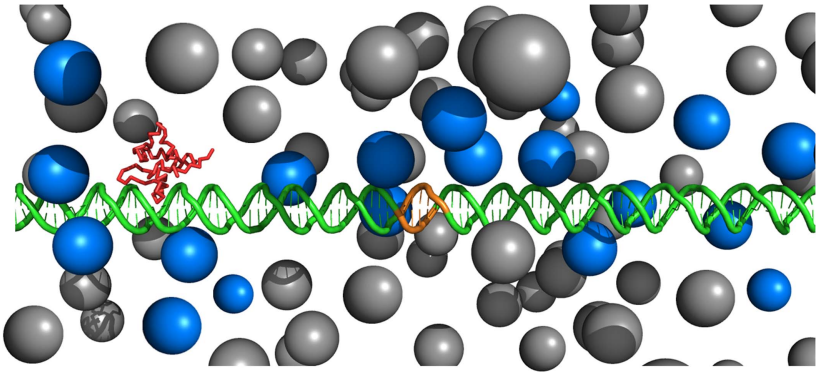

Figure 1. Schematic of DNA search by a protein in the presence of crowding particles. The DNA-binding protein and the DNA strand are shown in red and green respectively. The crowding particles are a mixture of those exerting only an entropic effect (i.e., excluded volume effects, gray spheres) and those that have affinity to the DNA (blue spheres). Two types of crowding particles with affinity were used: charged particles and particles with a Lennard–Jones (LJ) potential (they not differentiated from each other in the figure). The crowding condition is characterized by the volume fraction occupied by the crowding particle, φ, and the number of particles with affinity (either point charge or LJ potential), φ^*. Affinity between the charged particles and DNA may affect the characteristic time to localize the target site, τ^{Target} (marked in orange on the DNA strand). The crowding condition shown in the figure corresponds to a fractional volume of $\varphi = 50\%$, with charged particles of $\varphi^* = 20\%$.

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 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 [2–7]. The search dynamics, which is governed by non-specific protein–DNA interactions, is dominated by electrostatic forces and is therefore strongly dependent on the salt concentration, which influences partitioning between the 1D and 3D search modes of the facilitated diffusion process. At low salt concentrations, the electrostatic interactions between the DBP and DNA are stronger, and the protein mostly diffuses along the DNA major groove using a relatively short bi-directional 1D random walk along the linear contour of the DNA ('sliding') combined with short-range jumps between neighboring DNA segments ('hopping'). While both sliding and hopping are considered linear diffusion along the DNA, only in the sliding dynamics the protein follows the major groove and performs rotation-coupled translation dynamics. 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 [6]. In particular, jumping between distant DNA

segments via the 'Monkey-bar' mechanism [8–11] may improve the search efficiency. This mechanism was also analyzed using Lévy flight dynamics [12–15] and its high efficiency may suggest that it is preferred evolutionarily, at least when fast search is essential for function.

The physics and biochemistry of the facilitated diffusion mechanism have been investigated from both the theoretical and experimental perspectives, including through complex kinetic models [3, 16–19], simulation tools (both at the coarse-grained and atomistic levels) [20–26], and *in vitro* biochemical measurements at the bulk and single molecule levels [5, 27–29]. However, when such a search mechanism takes place in a crowded cellular environment, the positions of nucleosomes, and high concentrations of macromolecules and other proteins impose a physical constraint on the search process [30–37] (figure 1). Given that the macromolecules occupy 10%–40% of the total cellular volume (which corresponds to a concentration of ∼100–300 mg ml^{-1} [31, 38]), they are expected to have a non-negligible effect on search kinetics. Although some aspects of DNA search *in vivo* have been investigated experimentally [39, 40] and some from the theoretical perspective [41–46], much remains to be understood, particularly in the context of the crowded cellular environment.

Investigations of the effect of molecular crowding on the kinetics of protein–DNA interactions have shown that facilitated diffusion is affected by the cellular conditions [47–49]. 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'. The inter-molecular forces that expel small particles from between the larger molecules and thereby cause the exclusion effect are referred to as depletion forces. A recent study suggested that a crowded cellular environment could enhance the mechanism of DNA search. By creating low-viscosity micro-environments around the searching enzyme and DNA, crowding increases the likelihood that the enzyme will successfully translocate between its respective target sites without dissociating into the bulk solution [47].

Using coarse-grained molecular dynamics (CGMDs) simulations, it was recently demonstrated how molecular crowding influences the dynamics of DBP as it searches DNA [1]. DBP search efficiency may improve in the presence of crowding as search 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 an accessible region of the DNA. Search efficiency is also strongly affected by varying volume occupancies, which are in turn strongly dependent on salt concentration. It is suggested that, at higher 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.

In contrast, crowding molecules can also act as obstacles that block the 1D dynamics of the searching protein along the DNA and thereby truncate its sliding track into short fragments [44]. This may imply that an inhomogeneous positioning of obstacles (roadblocks) along DNA can sequester sampling to within certain regions while reducing the possibility of searching other regions and may result in a slower search when the target site is not located in

the accessible region defined by the obstacles [44, 50, 51]. Intuitively, mobile obstacles are expected to have a smaller effect on the search speed than static ones [45].

In addition to the fractional volume occupied by the crowding particles, the effect of crowding on diffusion along DNA may depend on the molecular properties of the crowding particles. We have shown that the dimensions and the mass of the crowding particles may modulate the effect of crowding on searching dynamics [1].

In the current study, we investigate the effect of crowding by molecules that have an affinity to the DNA and to the searching protein. This is obviously a more realistic situation than crowding particles that make a solely entropic (i.e., excluded volume) contribution. By introducing affinity between crowding particles and the DNA chain, this paper explores the effects 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 DNA search. By studying the different intrinsic dynamics of the different types of affinities of crowding macromolecules, we aim at providing a proper account of the crowded cellular environment, which is crucial for a full understanding of protein–DNA target search.

Materials and methods

In the CGMD simulations, the protein was represented by a single bead per residue placed at the Cα position and the protein dynamics was governed by its native-state topology [52, 53]. The DNA was modeled by three beads per nucleotide (representing phosphate, sugar and base) that were positioned at the geometric center of the represented group. We modeled a 100 base-pairs (bp) double-stranded DNA (ds-DNA) as a straight rigid molecule (about 333 Å) in its canonical B-form that was placed at the center of a cubic, size of 450 Å in each direction and was aligned with the *Z*-axis. The crowding macromolecules were represented by spheres that were initially located at random positions and that occupied a total volume of $\varphi = 50\%$ as determined by their number ($N = 1800$) and their radius ($R = 18$ Å). The DBP, DNA, and the crowding molecules interacted with each other via exclusion volumes. To mimic the more realistic scenario in which the crowding particles represent other proteins that may also interact with the DNA, affinity was added between the crowding particles and the DNA (see figure 1). Thus, the crowded environment was a mixture of particles with entropic and energetic effects.

The volume fraction, φ, corresponds to the total volume fraction of the crowders, while φ^* indicates the volume fraction of the particles that make an energetic contribution. Two types of crowding particles with affinity were introduced: (a) crowding particles with a point charge of $+1$ each; (b) crowding particles that can form a Lennard–Jones (LJ) interaction with the base bead of the DNA (with $\varepsilon = 1$ and an optimal distance of 22 Å). In this study, φ was set to be 50% and φ^* ranged between 0% and 20%. The radial distribution function (RDF) was used to determine the density and organization of crowding particles around a specific crowding particle as a function of distance.

We performed CGMD simulations of a human DBP Sap-1 (PDB code: 1bc8), in the presence of a 100 bp ds-DNA molecule of poly-GC. Sap-1, a 93 amino acid globular protein with a total of 15 and 6 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 non-native interactions and used the LJ potential to represent native contact interactions. Electrostatic interactions acting between all the charged beads in the system were modeled by the Debye–Hückel potential [6, 54].

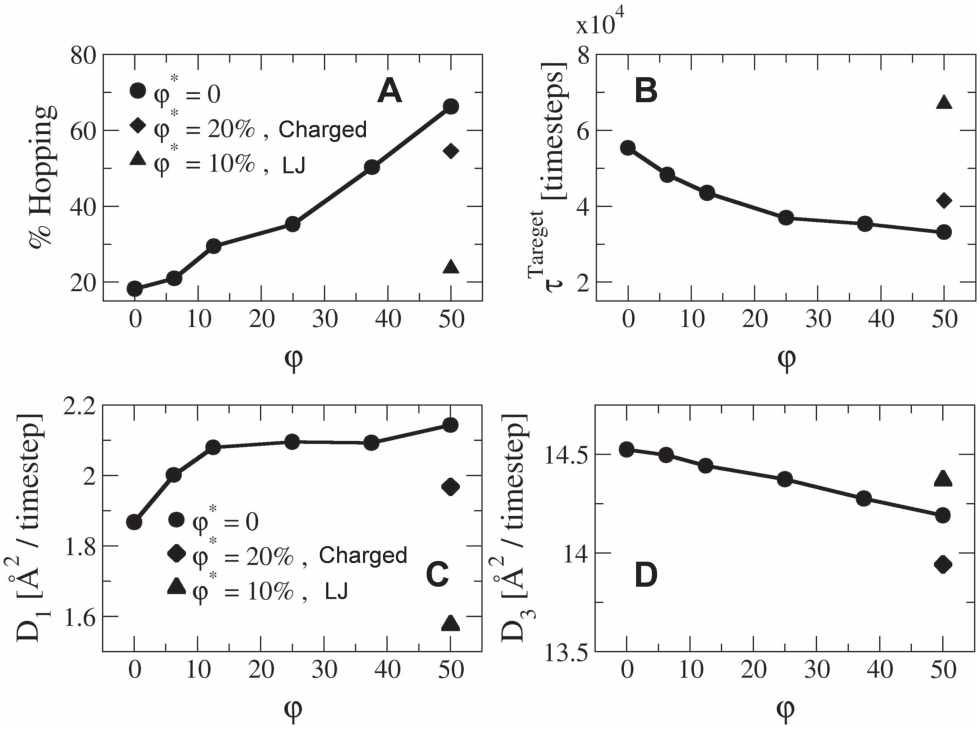

Figure 2. The effect of crowders with affinity to DNA on the mechanisms a protein uses to search DNA at a salt concentration of 0.075 M. The search mechanism is analyzed in terms of the usage of the hopping search mode (A), the mean time, τ^{Target}, needed to approach the target site when starting from a selected position on the DNA (B), the linear diffusion coefficient D_1 (C), and the 3D diffusion coefficient D_3 (D). The effect of volume fraction, φ, of entropic crowders (i.e., where $\varphi^* = 0$) on the search properties is shown by circles (data from [1]). The diamond and triangle correspond to crowders exhibiting a mixture of entropic and enthalpic effects, with crowders with a positive point charge present at a volume fraction of $\varphi^* = 20\%$ and crowders with Lennard–Jones potential present at $\varphi^* = 10\%$, to produce a crowding environment of $\varphi = 50\%$.

The dynamics of the protein along the DNA was simulated using Langevin dynamics [6, 55]. The simulations were performed at a constant temperature below the unfolding temperature of Sap-1 (i.e., $T_{\text{simulation}} \sim T_{\text{f}}$, 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 to allow extensive DNA sampling by the protein and transitions between sliding, hopping, and 3D search modes. The protein was considered to be utilizing the 3D diffusion mode if the center of the recognition helix was located more than 32 Å from the center of the closest DNA base pair. A snapshot was defined as taking part in a sliding search if at least 70% of the recognition region was in contact with the DNA major groove, the distance of the center of mass of the recognition region from the center of the closest DNA base pair was up to 12 Å longer than that in the crystal structure, and the orientation angle was less than 25°. If the recognition helix was found at a distance of less than 32 Å from the DNA and did not meet any of the criteria for the sliding mode, the frame was defined as representing protein hopping along the DNA. This definition allows clear differentiation of the search mode adopted by the DBP

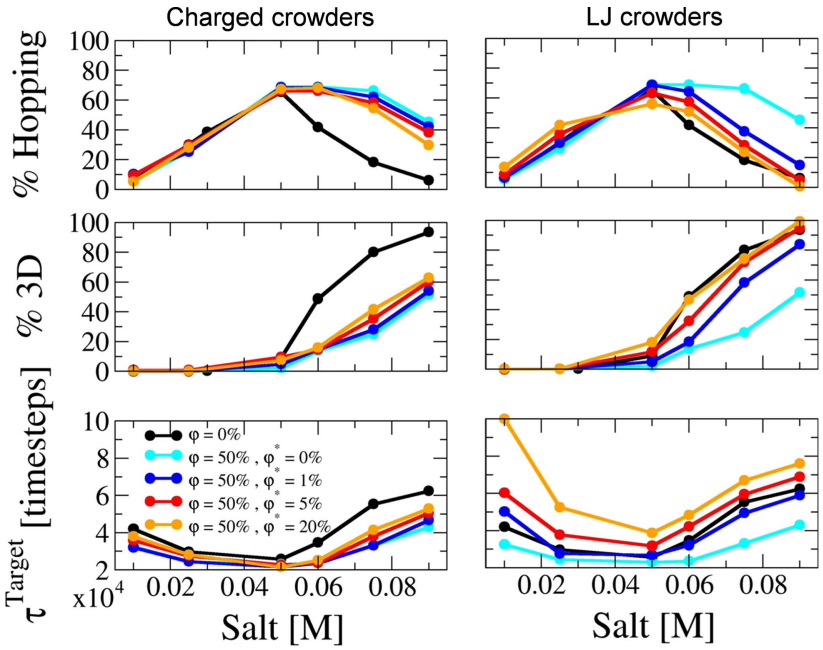

Figure 3. The effect of crowders with affinity to DNA on the mechanisms a protein uses to search DNA in environments having different ionic strengths. Affinity, φ^*, is achieved by introducing positive point charges of $Q = 1$ (left panels) and by introducing Lennard–Jones (LJ) interactions of $\varepsilon = 1$ (right panels) between the crowding particles and the DNA chain while varying the salt concentration. The graphs show the effect of affinity on the usage of: the hopping search mode (top panels), the 3D diffusion (middle panels), and the kinetics of DNA search (bottom panels) at various salt concentrations. Here, τ^{Target} indicates the mean time required for the searching protein to approach the target site when starting from a selected position on the DNA strand.

during its interactions with the DNA while it interacts with DNA [6]. 1D diffusion along the DNA was used to calculate the mean-square displacement profiles along the *Z*-axis [6, 56]. 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.

Results and discussion

In this study, we examine how introducing DNA affinity to the crowding particles may affect their DNA search kinetics compared with that of crowders that exert only an entropic effect. Two types of crowding particles with affinity to DNA were considered: crowders with a point charge of $+1$, and crowders that can form a LJ interaction with the DNA. While the former crowders are influenced by salt concentration, the latter are insensitive to salt and are expected to interact with the DNA more tightly because of their short range nature. The positively

charged crowders also differ from the LJ particles in that they may interact not only with the searching protein but also with each other. The effect of crowding particle affinity to DNA on DBP search dynamics can be elucidated by examining changes in the search protein's usage of the sliding, hopping, and 3D diffusion search modes under various conditions. Previous studies have shown that the search efficiency of a DBP is largely dependent on salt concentration, and also on the fractional volume of crowding particles [1] and therefore these are the conditions we varied.

We have previously shown [1] that crowding particles that exert an entropic effect may speed up the search kinetics by increasing the search protein's usage of the hopping mode at the expense of 3D diffusion (figure 2(A)). The entropic effect exerted by the crowding particles on the diffusing protein is associated with a reduction in the number of dissociation events of the searching protein from the DNA and with faster linear diffusion (figure 2(C)). These effects increase as the fractional volume of the crowders, φ, increases and are accompanied by fast search kinetics (as measured by τ^{Target}; figure 2(B)). A larger effect of crowding is observed at higher salt concentrations, which are also those in which searching via the hopping mode is more accessible (figure 3).

Since the crowding effect is larger at higher fractional volumes [1], we studied the effect of crowders with affinity for cases in which $\varphi = 50\%$. A fraction of the crowding particles were converted from purely entropic particles to crowding particles with affinity to DNA (charged particles or particles able to participate in LJ interactions). The fractional volume of the particles with affinity, φ^*, was 0%–20%.

We began by examining the effect of crowders with positive charges on the dynamic modes of the systems, focusing on the usage of the hopping search mode (figure 3, upper left panel) and 3D (figure 3, middle left panel). A profound effect of the positively charged systems on both the hopping and 3D search modes is observed at higher salt concentrations, with the latter occurring at the expense of the former. This shift in favor of the use of 3D diffusion can be attributed to the fact that under such conditions both the positively charged crowding particles and the DBP are mostly bound to the DNA. This effectively reduces their role as crowders, so resulting in a weaker excluded volume effect that further weakens as φ^* increases, while increasing their role as road blocks by increasing the number of obstacles lying between the searching BDP and its target site. The time to localize the target sites, τ^{Target}, increases together with φ^*. Thus, the presence of crowding particles with greater DNA affinity results in slower search kinetics. This result suggests that the differences in search time for various degrees of affinity stem from the different mobilities of crowded systems having various values of φ^*.

We now move on to describe the effects of crowding particles whose affinity stems from their ability to form LJ contacts with DNA. In LJ affinity, each particle is uncharged, yet is able to form a contact with each of the 100 sites along the DNA. Thus, repulsion forces between the crowding particles are retained, so maintaining the exchange of particles at the DNA sites. Due to the limited number of accessible DNA sites, it should be noted that the overall values of φ^* for the LJ crowders are smaller than those for the charged crowders.

Compared with the charged crowding particles, LJ crowders have a more profound effect on partitioning between the search modes. The formation of a short-range LJ contact may reduce the extent to which hopping dynamics are typically enhanced by crowding conditions. Upon increasing the fractional volume of the LJ crowding particles (at higher salt concentrations), the DBP simply escapes the DNA and performs 3D diffusion (figure 3, middle left panel), which resembles the situation of DNA search in the absence of crowding. The stronger effect of the LJ crowding particles compared with that of charged particles originates

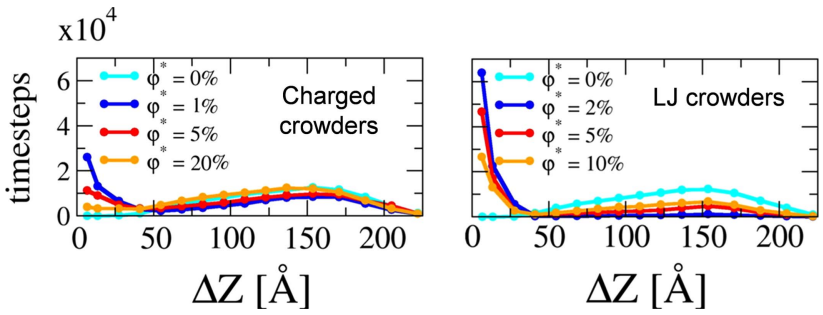

Figure 4. The mean residence time of crowders with DNA affinity at different distances from the Z axis of the DNA molecule. The distributions for charged crowders with $Q = 1$ (left panel) and Lennard–Jones (LJ) crowders with $\varepsilon = 1$ are shown for different values of φ^*.

from their tighter interaction with the DNA. The LJ crowding particles are more static, thus their effect more resembles that of a static roadblock.

The effect of the LJ crowders on the kinetics of the systems results in even slower kinetics compared with diffusion in the absence of crowding (figure 3). Generally, even at a low φ^* of 1%, the effect on kinetics is profound for all salt concentrations. At a 0.01 M salt concentration, the DBP undertakes a linear search via 1D sliding, however, its pathway is restricted due to the LJ crowding particles and it is unable to perform effective hopping events. At moderate salt concentrations, the DBP performs more hopping and 3D diffusion, therefore in the presence of a small numbers of blockers (such as $\varphi^* = 1\%$) it is still able to find its target. Upon further increasing the salt concentration, the DBP performs more 3D diffusion at the expense of hopping events, and ultimately escapes the DNA into the bulk. Increasing the value of φ^* results in an increase in τ^{Target} at all salt concentrations because LJ crowders occupy many of the DNA sites and thus the DBP can reach its target site only following the dissociation of the LJ crowders (for example, following collisions with the protein or other crowders). As φ^* increases, the dissociation of an LJ crowder is likely to be followed by the association of another LJ crowder in its place, thus resulting in a higher τ^{Target}.

To further examine differences in the effect exerted by charged compared with LJ particles on DNA search by a DBP, we analyzed the physical characteristics of the linear diffusion component of the search, given that linear search modes dominate at low to moderate salt concentrations. Figure 2(C) shows the 1D diffusion coefficient (D1) for various fractional crowder volumes at a salt concentration of 0.075 M, at which the influence of crowding is most pronounced (above such salt concentrations, the protein performs mostly 3D diffusion in the bulk). As can be seen, introducing positively charged crowding particles (diamond shape) results in a decrease in the D1 diffusion coefficient. The even lower D_1 caused by adding LJ crowders with affinity to DNA is consistent with the DPB's lower usage of the hopping search mode (in favor of 3D diffusion) that is observed when LJ crowders replace some of the entropic crowders (figure 2(A)) and further exemplifies the roadblock effect exerted by LJ crowders (figure 2(C)). Consistently with the pronounced decrease in the proportion of hopping with increased crowding (figure 2(A)), this decrease becomes greater at higher salt concentrations.

In addition, we examined the effect of molecular crowding on 3D diffusion in the bulk (D_3) (figure 2(D)) for different fractional volume values (circle shapes). D_3 is lower in the

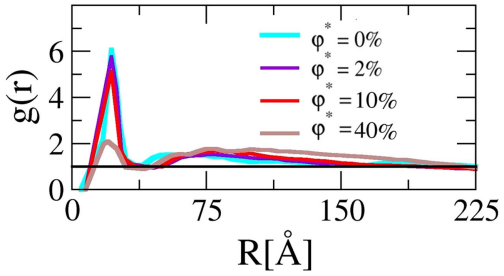

Figure 5. Radial distribution function of single particles moving in a three-dimensional box as a function of the radius (R) for various degrees of affinity, φ^*.

case of positively charged crowders (diamonds) than entropic crowders (triangles). This can be the result of an increase in the excluded volume due to multiple collisions between two positively charged particles, suggesting that, for large values of φ^*, the movement of the DBP is more confined when it is located in the 3D bulk and that its 3D diffusion is slower in this viscous environment. The frequency of collisions between the crowding particles is similar for LJ and entropic crowders. The LJ crowding particles are concentrated in the vicinity of the DNA, which results in a decrease in the excluded volume around the DBP in the bulk. Therefore, in such systems, an opposite effect is achieved with a small increase in D_3 compared with the situation in the presence of positively charged or entropic particles.

To better understand the origin of the lower efficiency of the crowding particles in enhancing the facilitated diffusion of the searching protein upon changing their properties from solely entropic to including an enthalpic component, we consider that the crowding particles do not only differ in their interactions with the DNA but also in their internal dynamics. Figure 4 shows the distribution of the location of the particles from the DNA axis (Z) for systems with different values of φ^*. We compare these distributions to the corresponding distribution for uncharged crowding particles that move freely in the box with restrictions due only to repulsive interactions with other molecules.

The entropic crowders ($\varphi^* = 0$) are evenly distributed within the simulated box. The overall shape of this distribution is changed for crowders with affinity because these particles are attracted by the negatively charged phosphate groups of the DNA (figure 4). While a sharp accumulation of positively charged crowders is seen for ($\varphi^* = 1\%$), a more moderate trend is seen for higher values of φ^*. The reduced population of charged particles around the DNA when their concentration increases is likely due to electrostatic repulsion between positively charged particles resulting in their collision. The overall high mobility of such a system ultimately results in a decrease in the extent to which each particle locates close to the DNA. However, due to the large number of charged particles, the overall number of charged crowders that interact with the DNA increases, ultimately restricting the ability of the DBP to reach new DNA sites, thus explaining the inhibited nature of its kinetics and search modes, as was shown in figures 2(B) and 3. By contrast, LJ crowding particles tend to accumulate on the DNA and their residence time is much longer than that of the charged particles.

In figure 5, we compare the RDF of systems of charged particles with different values of φ^*. For this, we examined the distribution of a system containing solely crowding particles confined in a box (without the DBP and the DNA strand), and analyzed the distribution around particles closest to the center of the box. When examining the distribution of the uncharged system ($\varphi^* = 0$), the first distribution peak is located between ($18\text{Å} \leqslant Z \leqslant 36$ Å) corresponding to the radius of the crowding particles. Increasing R results in a broader peak

that decays throughout the box until reaching $g(r) = 1$ near the edges of the box. Introducing positive charges to the particles (increasing φ^*) results in an increase in the distribution throughout the box, at the expense of the peak close to the DNA. This suggests that the collisions between the positively charged particles disrupt the natural arrangement of the crowding particles, increasing the distribution of particles at longer distances. This further exemplifies how increasing φ^* results in less crowding close to the DNA, which affects DBP dynamics, further explaining the results obtained in figure 3.

Conclusions

In addition to the typical complexities of biomolecular recognition, which demand structural and chemical complementarity, the interactions of a protein with DNA require the former to undertake an extensive search that is further complicated by conditions in the cellular milieu. Surprisingly, several studies [44, 46, 47, 50] have suggested that cellular complexity may not significantly exceed the *in vitro* scenario. It was reported that crowding molecules in the bulk can enhance facilitated diffusion by decreasing the dissociation constant of the protein from non-specific DNA and by accelerating linear diffusion [1]. Particularly, crowding on the DNA (by roadblocks) may not necessarily impede the search kinetics [44, 46] as these obstacles might be bypassed under some conditions.

The molecular properties of the crowders and roadblocks may dictate their effects on the search mechanism. The dimension of the roadblocks, for example, may affect the ability of the searching protein to bypass them [44]. Similarly, mobile roadblocks are expected to slow down the search kinetics to a lesser extent than static obstacles [44]. Furthermore, important parameters influencing the characteristics of linear diffusion include not only the entropic effect they exert via excluded volume interactions but the mass of the crowding particles and their size. At a constant fractional volume, decreasing the mass of the crowding particles or decreasing the dimension of the crowding particles increases the number of particle–protein collisions. Consequently, the linear diffusion of the DBP along the DNA will be characterized by a higher D_1 due to faster sliding or due to larger usage of the hopping searching mode. The effect of entropic crowding and of obstacles provides evidence that D_1 can be affected not only by the salt concentration (which changes the hopping propensity) but also by other features of the cellular environment.

In this study, we examined how properties of the crowding particles other than their size and mass may affect the mechanism by which a searching protein localizes its DNA target site. We introduced crowding particles with affinity to the DNA to mimic a more realistic crowded environment that has not only excluded volume interactions but also an enthalpic component. We used two types of crowders that interact with the DNA either via electrostatic or LJ interactions. One may view these crowding particles as having characteristics of both crowders and roadblocks because they diffuse in the 3D bulk and can also transiently interact with the DNA. Additionally, molecular crowding may affect the biophysics of DNA search in other ways than manipulating D1 and D3. For example, molecular crowding is expected to change the efficiency of the 'Monkey-bar' mechanism or the transition from nonspecific to the specific binding modes.

Using this approach, we aimed at evaluating the relative contributions of crowding particles and obstacles to DNA search by a DBP. We found that adding crowder affinity to DNA reduced the efficiency of crowding as a search facilitator that increases 1D diffusion via hopping, and therefore reduced D_1 and increased the search time. The more transient the

interactions of the crowders with the DNA (i.e., the greater the extent to which they acted as mobile obstacles), the larger their effect on search efficiency.

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