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Facilitated Diffusion of Transcription Factor Proteins with Anomalous Bulk Diffusion

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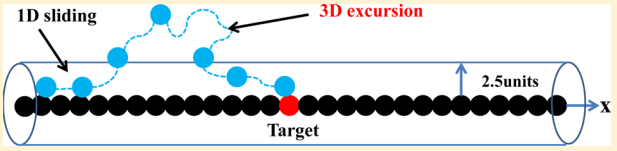
ABSTRACT: What are the physical laws of the diffusive search of proteins for their specific binding sites on DNA in the presence of the macromolecular crowding in cells? We performed extensive computer simulations to elucidate the protein target search on DNA. The novel feature is the viscoelastic non-Brownian protein bulk diffusion recently observed experimentally. We examine the influence of the protein−DNA binding affinity and the anomalous diffusion exponent on the target search time. In all cases an optimal search time is found. The relative contribution of intermittent three-dimensional bulk diffusion and one-dimensional sliding of proteins along the DNA is quantified. Our results are discussed in the light of recent single molecule tracking experiments, aiming at a better understanding of the influence of anomalous kinetics of proteins on the facilitated diffusion mechanism.



INTRODUCTION

The Berg-von Hippel model of facilitated protein diffusion was set forth in a series of seminal works in the 1970−80s,[1−7] following experimental proof that transcription factor proteins, such as lac repressor,[8] are capable of localizing their specific DNA binding sites remarkably fast: the association rate constants k_{on} of lac repressor to its operator site reach $k_{on} \sim 10^{10}/(\mathrm{M} \times \mathrm{s})$ at salt levels of $\lesssim$0.1 M,[1,6] $\sim 10^2$ times faster than the Smoluchowski diffusion limit.[9−12] At higher salt concentrations, the rate of protein−DNA association rapidly decreases, indicating diminished lac-DNA electrostatic attraction.[13,14] In facilitated diffusion, the proteins undergo alternating rounds of 3D bulk excursions and 1D sliding along DNA, to speed up the target localization.[11,12,15−29,31−38] Proteins may also perform long jumps or transfers between distant DNA segments,[4,24,25,39] further optimizing the search.[12,19,20,40]

The recognition of specific binding sites by regulatory proteins is believed in some cases to proceed as a two-step process or involve two different protein binding states, in order to resolve the speed-stability paradox;[16,19,20] see ref 41 for the conditions of its existence. In the two-step models, the protein first binds to DNA nonspecifically, e.g., by weak electrostatic contacts, staying in adjustable conformations. Once the specific binding site is localized, some protein−DNA adjustment occurs and effects a stronger, specific protein−DNA binding, protein shape adjustment or folding,[15] and protein−DNA site recognition (often via hydrogen bonds[8]). For two-state models, the protein switches its conformation either randomly or as a response to signals[35] ensuring that sliding along the DNA occurs fast enough, at the same time enabling strong enough cognate site recognition. In the past decade, a series of experimental and theoretical advances[11,12,17−38,42−44] allowed to unravel important features of protein diffusion on DNA and DNA−protein interactions: effects of DNA conformations, DNA internal dynamics, sequence specific binding, protein conformational dynamics, target antenna, spatial colocalization, stochastic concentration fluctuations, and hydrodynamics were investigated.

In contrast to classical experimental and theoretical studies, effects of molecular crowding, both in the solution and on DNA have come into focus only recently.[31,36,45−53] Crowding in the cell cytoplasm[54−59] and by "road blocking" on the DNA can either impede or facilitate the target search by proteins.[49,52] The long-time diffusion of proteins and other tracers in biological cells can be slower than Brownian,[54−57] with the particle mean squared displacement (MSD) featuring anomalous scaling,[58,60,61]

$$\langle \mathbf{r}^2(t) \rangle \simeq t^{\alpha} \tag{1}$$

The motion of submicron beads in crowded fluids was shown to be consistent with fractional Brownian motion (FBM).[62,63] In vivo, this viscoelastic diffusion of particles depends on the cell phase and position inside the cell.[64−67] The range of measured exponents α is quite large (see Tabs. 2, 3 of ref 58). For proteins, the range was measured to be $\alpha \sim 0.6\cdots0.9$,[55,56] also consistent with findings of protein diffusion in the crowded *E. coli* cytoplasm via explicit large-scale Brownian Dynamics simulations.[59] The study[58] also provides an overview of mathematical models of anomalous diffusion in crowded biological cells. The exponent α tends to decrease at higher concentration of crowding molecules, for larger tracers, and in more viscous fluids.[54,56,58,63] In crowded media, the association rates of closely positioned proteins—as well as of the ends of a polymer chain[68]—can, in contrast, get

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facilitated by crowding due to "caging". The effects of crowding on molecular diffusion and reaction rates in gene regulatory biochemical networks were considered in detail.[69]

Complementing similar approaches to protein search in eukaryotic nuclei using geometrically restricted diffusion,[53,70] we here employ Langevin Dynamics simulations to study the target localization dynamics by DNA binding proteins via viscoelastic anomalous protein diffusion. We rationalize the implications of the anomalous bulk diffusion within the Weierstrass−Mandelbrot FBM framework (WM-FBM). We start from a description of the simulations model and the approximations employed, before presenting the main results. We conclude with a discussion of possible applications and a critical evaluation of our findings.

MODEL AND APPROXIMATIONS

We employ a simple model of protein diffusion, considering a single target on a $L = 71\sigma$ long stretch of straight rod-like DNA positioned in the middle of the simulation box of volume $V = 50 \times 50 \times 75\sigma^3$.[49] The protein diffusion in the bound state follows the Langevin equation (LE), $m d^2\mathbf{r}_i(t)/dt^2 = -\sum_j \nabla U_{LJ}(r_{ij}) - \bar{\xi} d\mathbf{r}_i(t)/dt + \mathbf{F}_i^R$, as developed in refs 48,49. Here the Lennard-Jones potential of depth ε_0 is $U_{LJ}(r) = 4\varepsilon_0 [(\sigma/r)^{12} - (\sigma/r)^6] + \varepsilon_0$ for $r \le 2^{1/6}\sigma$ and 0 otherwise.

A single protein of size σ and mass m starts at the end of the DNA within the binding zone of 2.5σ, Figure 1. Other initial conditions can be a random placement of proteins in the simulation box or on its boundary, mimicking a given protein level in the bulk. The search time behavior can also differ for varying box size and DNA length L. A detailed analysis of these effects is however beyond the scope of this work. We here want to study the immediate effects of anomalous bulk diffusion in comparison to the earlier studies[48,49] with analogous boundary and initial conditions. We set $\bar{\xi} = 0.7$ for the friction coefficient, $k_B T = 1.2\varepsilon_0$ for the thermal energy,[48] with the Gaussian random force $\mathbf{F}_i^R$ acting on the ith particle. One time step in our simulations—if applied to the tetrameric lac repressor—is $t_{LJ} = \sqrt{m\sigma^2/k_B T} \approx 2.2$ ns, in terms of ε_0, σ and m parameters.[71] The LE damping is so that 1D diffusion of proteins stays ballistic for the first $\sim 10^2$ steps, after which the transition to the Brownian behavior takes place (MSD results not shown).

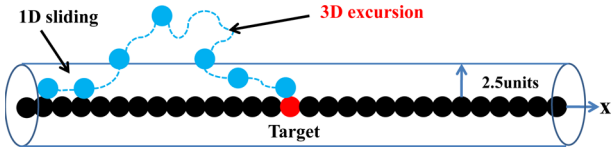

Figure 1. Schematic of protein diffusion: the proteins perform an intermittent search combining 3D bulk diffusion and 1D sliding on the DNA to locate their specific binding site.

The protein performs multiple rounds of attachment to and detachment from the DNA, with binding energy $\epsilon > 0$. The critical binding radius to DNA defines the range of the protein−DNA interaction potential, given by the cutoff distance $r_c = 2.5\sigma$ of $U_{LJ}(r)$. When the target is detected the search process is stopped, the protein is annihilated and reintroduced again at the chain's end.[48,49] Other starting conditions of proteins—such as a uniform distribution on the cell boundary or in the simulation box—will affect the search time, in particular at conditions when the number of 3D-1D diffusion rounds is small. In contrast, for rather weak protein−DNA binding energies—when multiple binding-unbinding protein−DNA events are expected to occur before the target is found—the effect of the initial position should be rather weak: 3D excursions destroy the memory about the starting point. This most realistic scenario is the focus of the present study. Moreover, as stated above, the present choice allows a ready comparison with the studies.[48,49] A detailed comparative analysis of these effects will be the focus of another study.

A protein starting position on the chain's end was also used previously by one of the authors (L.L.) for the Langevin dynamics of proteins on DNA.[48,49] Thus, the results of FBM and LE in the bulk for the search time can be compared at the same starting conditions. The LE is integrated with the method of ref 72, similar to the implementation in refs 48,49. The periodic boundary conditions applied in simulations in x, y, and z directions, are also similar to refs 48,49. In our model, bulk crowding is taken into account effectively as viscoelastic anomalous diffusion of the FBM type, corresponding to the overdamped viscoelastic generalized LE.[60] No site-to-site barriers for protein diffusion along the 1D DNA chain exist in the current model, see refs 19,35,73,74 for possible implications of such barriers on the protein kinetics.

To simulate the bulk FBM protein motion, consistent with diffusion of molecules in a crowded cell,[62] we implement the computational procedure proposed in refs 75−77 for the particle positions $x_k(t)$ in three dimensions $k = \{1, 2, 3\}$ and use the forward LE, $x_k(t + 1) = x_k(t) + \xi_k(t)$. The increments of the particle displacements, $\xi_k(t) = W(t + 1) - W(t)$, are described in terms of the Weierstrass−Mandelbrot function[76,77]

$$W(t) = \sum_{n=-\infty}^{n=+\infty} \frac{\cos[\phi_n] - \cos[\gamma^n t^* + \phi_n]}{\gamma^{n\alpha/2}} \qquad (2)$$

Here α corresponds to the anomalous diffusion exponent in the MSD (1).[78] The values ϕ_n are random phases in the interval $(0, 2\pi)$. We set $\gamma = \sqrt{\pi}$ and restrict the sum in eq 2 to $-8 < n < 48$.[75,77] The desired length of the simulated trajectories is fixed, so that $t^* = 2\pi t/t_{max}$ where $t_{max} = 10^5$ is the total number of simulation steps, $t = 1, 2, \ldots, t_{max}$. This parameter affects the random numbers generated via the algorithm (eq 2).

The WM-FBM procedure enables the simulation of subdiffusive motion with $\alpha \approx 0.5\cdots0.9$, relevant to protein diffusion in cells.[54,56,57] We tested that this simulation approach yields the correct MSD scaling for free diffusion, Figure 2. Note, however, the spurious periodic oscillations of the MSD stemming from the trigonometric functions in eq 2, see also Figure 7A in ref 77. Despite this subtlety, the WM-FBM approach is widely used for its computational efficiency. In Figure 2 the MSD curves are shifted vertically: to avoid the crossing of curves the results for $\alpha = 1$ and 0.7 were multiplied by respective constants so that all MSD asymptotes start at the same point. In our FBM simulations according to the scheme (eq 2) the generalized diffusion coefficient D_{3D} is not varied and for the target search computations no shifts of particle positions—such as those for the MSDs in Figure 2—were performed. The 1D protein diffusivity is set for simplicity identical to that in the bulk, $D_{1D} = D_{3D}$, enabling us to focus on the effects of anomalous bulk transport of proteins. In a more realistic biochemical description, the value of 1D protein diffusivity will likely be correlated to its DNA binding energy.[11,20]

MAIN RESULTS

Figure 3 shows the results for the mean search time $\langle t \rangle$ for the target. It is proportional to the average number $\langle N_R \rangle$ of rounds of 1D and 3D protein diffusion,

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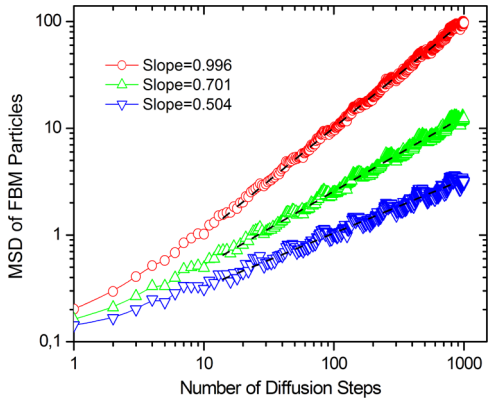

Figure 2. MSD of FBM particles in 3D for exponents $\alpha = 0.5, 0.7, 1$. The curves are shifted vertically for convenience so that the long time MSD asymptotes (dashed lines) hit the same point at $t = 1$. The averaging is performed over $N = 2000$ trajectories and the dashed asymptotes are fits to eq 1.

Figure 3. Total mean search time $\langle t \rangle$ versus protein−DNA binding strength ϵ, plotted for different exponents α. The number of traces used for averaging is $N = 2000$, except for $\alpha = 0.5$ with $N = 10^3$ due to computational expenses. The error bars are often smaller than the symbols. The simulation time for each curve is ∼50 h on a standard work-station. Longer simulations are needed as α decreases, as one can judge from the size of the error bars. Note a Boltzmann-like growth of $\langle t \rangle$ with ϵ at strong protein−DNA binding.

$$\langle t \rangle = \langle t_{1D} \rangle + \langle N_R \rangle (\langle t_{3D} \rangle + \langle t_{1D} \rangle) \quad (3)$$

We find that $\langle t \rangle$ varies nonmonotonically with protein−DNA binding strength ϵ. This is a known feature for Brownian facilitated diffusion in solution[11,16,50] based on solid experimental support.[3−6] A simple physical reasoning for this $\langle t(\epsilon) \rangle$ nonmonotonicity goes as follows. For weak binding strengths, the adhesion of proteins to DNA is not sufficient to establish an optimal concentration of DNA-adsorbed proteins. The proteins diffuse in solution most of the time and rarely find the target via a direct binding to DNA. In contrast, for strong protein−DNA binding, the preference for the sliding phase is so large that a strong oversampling of, particularly long, DNA molecules occurs. At intermediate ϵ, an optimal combination of bulk protein relocations and 1D sliding on DNA is achieved so that the target association time attains its minimum. Such binding strengths corresponds to intermediate protein sliding distances along DNA, realized, e.g., at intermediate salt levels.[11,12,16,26]

Our results demonstrate that the nonmonotonic $\langle t(\epsilon) \rangle$ trend persists also for anomalous bulk protein motion. The effect for the optimal search time in fact becomes much more pronounced for more subdiffusive bulk diffusion. Moreover, the optimal target search time shifts to progressively longer times as the exponent α attains more pronounced subdiffusion, compare the curves in Figure 3 at high ϵ values. This is our first main result.

Analyzing the behavior of $\langle t \rangle$ for different α values, we find that—for weaker binding energies ϵ—the target localization time increases dramatically for more subdiffusive bulk protein motion, as intuitively expected. In contrast, for very strong protein−DNA binding the curves for different α values almost superimpose and the overall search time increases $\simeq \exp(\epsilon)$ with ϵ, Figure 3. The position of the minimum in Figure 3 shifts to higher ϵ values for smaller α, likely due to slowing down space exploration propensity because of stronger protein interactions with the medium. This is consistent with the increase of the effective binding constant in crowding conditions.[69]

Decomposing the total search time into 1D and 3D contributions, we find that the time a protein spends in bulk excursions drops dramatically with increasing binding strength and increases massively with decreasing α, see Figure 4A.

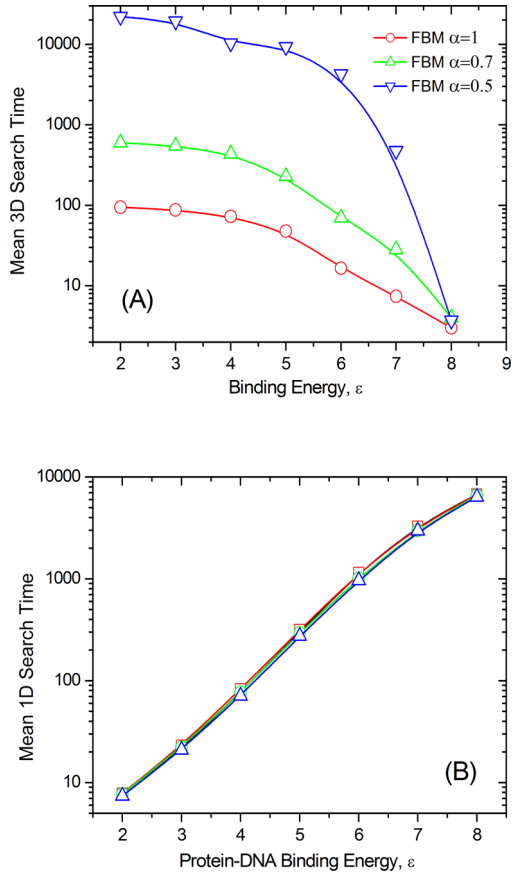

Figure 4. Average durations of one round of 3D (panel A) and 1D (panel B) diffusion versus the binding strength ϵ, plotted for the parameters of Figure 3. A weak dependence of 1D search times on α in panel B is within statistical uncertainties.

Note here that in the absence of protein−DNA binding, the mean search time in the bulk expected theoretically for Brownian diffusion is $\langle t_{3D} \rangle \sim V/(4\pi D_3 L)$. For the parameters used this yields $\langle t_{3D} \rangle \sim 360$, quite close to simulations results for $\alpha = 1$ at $\epsilon \to 0$, Figure 3. In contrast, the search time proteins spent sliding on DNA increases nearly exponentially with the protein−DNA binding strength ϵ, Figure 4B. This supports the standard Arrhenius kinetics, with protein−DNA unbinding rate

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$k_{off}(\epsilon) \sim 1/\langle t_{1D}(\epsilon)\rangle \simeq \exp[-\epsilon/(k_BT)]$. The 1D component of the search times presented in Figure 4B is nearly insensitive to the exponent α, as expected. Note that the sliding length l_{sl} of proteins along DNA[11,25] is also expected to grow exponentially with ϵ, $l_{sl}(\epsilon) \sim \sqrt{2D_{1D}\langle t_{1D}(\epsilon)\rangle}$.

For the optimal search conditions we observe that the times proteins spend in 1D and 3D diffusion modes are not equal, Figures 4A,B. This is in line with recent theoretical understanding[11] and different from models observing $\langle t_{3D}\rangle \approx \langle t_{1D}\rangle$ for immediate protein relocations (practically infinite D_3) in the bulk.[19,25] Note that single particle tracking experiments in living bacteria also revealed that at natural conditions lac repressor proteins can spend ~9/10 of time in the DNA-bound state.[18]

Figure 5 illustrates the number $\langle N_R\rangle$ of rounds of 1D and 3D diffusion (Figure 4) necessary to locate the target. We find that

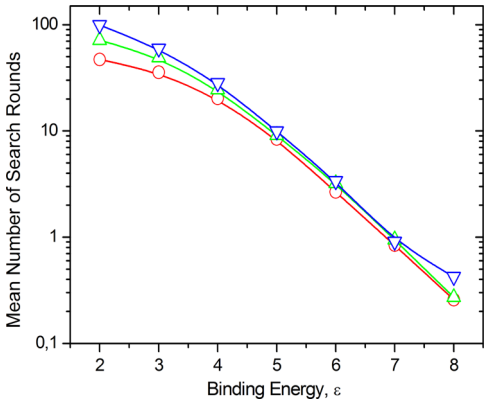

Figure 5. Average number $\langle N_R\rangle$ of rounds of protein diffusion necessary to locate the target, evaluated for the parameters of Figure 3. The notation for the curves is the same as in the previous figures. $\langle N_R\rangle + 1$ is the number of 1D sliding events.

(for a fixed DNA length) it decreases sharply with the strength of the protein−DNA attraction. For substantial protein−DNA attraction this decay is nearly exponential, inverse to the dependence of $\langle t_{1D}\rangle$ on the binding strength in Figure 4B. Thus, for progressively stronger protein−DNA binding less rounds of diffusion are required to find the target. Also—due to the antipersistent motion of FBM particles leading to more likely returns to already visited points for smaller α—the number of rounds $\langle N_R\rangle$ systematically increases for more subdiffusive bulk motion, see Figure 5. Although not a very pronounced effect, this is our second main result. Depending on different settings, this effect may well become more relevant. Note that at weak protein−DNA binding the 1D search time is very short, which might limit the applicability of the continuum Langevin equation in this regime.

Note that the average number of rounds can drop below unity at very strong protein−DNA binding, Figure 5. The reason is that the initial protein position is always at the end of DNA and the particle might not even leave the 1D mode once, corresponding to $N_R = 0$ and thus $\langle N_R\rangle < 1$, in accord with Figure 4.

DISCUSSION AND OUTLOOK

We presented results of computer simulations of protein search for a target on a stretch of rod-like DNA. The implications of crowding and protein subdiffusion in solution are our main focuses here. In the bulk, we used FBM to mimic a non-Brownian and subdiffusive motion of proteins. We demonstrated that the target search time features a pronounced minimum versus the strength of protein−DNA binding ϵ, for normal and subdiffusive motions. The optimal target search time is found to rise significantly as a function of ϵ for smaller exponents α. We characterized this increase of $\langle t\rangle$ as the bulk motion slows down due to a more local space sampling by FBM.

As a continuation of recent target localization studies in the presence of road blockers on DNA,[49,51] we plan to extend the current approach to crowded and partly nonaccessible DNA, possibly with mobile crowders.[50,52] Another biologically relevant modification is the target search when $D_{1D} \neq D_{3D}$, as experimentally observed[17,18] and theoretically described.[11,12,16] A finite concentration of a continuous semiflexible coiled DNA[26] with a proper statistics of loops can also be considered. Our simulations can be generalized to study facilitated protein diffusion on mobile polymer chains. This might affect the functional behavior of the search times, particularly at conditions of weak protein−DNA binding, when typical 3D search times of proteins can be comparable to characteristic polymer relaxation times. Lastly, recent single particle tracking experiments[40] indicate that multiple encounters of transcription factors and their cognate DNA sequences can be necessary before forming a specifically bound complex. This fact can be included in future models as well. Finally, we note that it may be important to unravel the full distribution of protein search times due to the few-encounter limit relevant for molecular signaling.[79]

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Notes

The authors declare no competing financial interest.

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