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And did he search for you, and could not find you?

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Abstract
We discuss stochastic search mechanisms for sparse targets. These mechanisms may be normal diffusion in different dimensionalities as well as anomalous diffusion, namely, Lévy walks and subdiffusion. We argue that under different boundary conditions either mechanism may be beneficial for the search. As a concrete example we investigate facilitated diffusion of regulating proteins in gene regulation. Moreover we discuss potential pitfalls in acquiring diffusion behaviour from time series analysis of single particle trajectories.

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(Some figures in this article are in colour only in the electronic version)

1. Introduction

As beautifully couched in this title quotation from William Shakespeare's comedy *The Merry Widows of Windsor* [1] a successful search requires (i) an efficient search method and (ii) a reliable detection mechanism. While lack of either leads to the failure to locate Sir John Falstaff in the buck-basket, many search processes in nature or even large digital databases successfully rely on these two fundamental principles, in particular, when the targets are rare. A prime example is the stochastic search of so-called transcription factors, DNA-binding

proteins, for their specific (cognate) binding site in gene regulation [2–5]: within megabases of DNA code such a transcription factor manages to bind to its cognate target site some two orders of magnitude faster than the three-dimensional Smoluchowski diffusion limit. This is ingeniously achieved by facilitated diffusion [6], the combination of different passive search mechanisms discussed below, whereby an intimate balance between search speed and faithful location of the binding sequence is attained [7]. Concentrating on the efficiency of the search we here discuss facilitated diffusion including the role of the polymeric conformations of the DNA in typical *in vitro* experiments under dilute solvent conditions, a situation encountered in numerous biochemistry experiments. We do not consider effects due to DNA packaging in biological cells. However we explore potential effects of reduced volume diffusion in a living cell under conditions of molecular crowding.

In a separate section we address in more general the principles of stochastic search for rare targets. We show that a Lévy walk strategy due to its scale freeness may be beneficial for the search for rare targets, and in addition makes the optimal search strategy more robust against changes in the environmental parameters.

2. Intermittent search and Lévy statistics

The trajectory of a Brownian random walk has the fractal dimension $d_f = 2$. In a one- or two-dimensional environment a Brownian walker is space filling and therefore prone to return to already visited sites [8]. We will refer to this tendency of many revisits to the same site as oversampling. In contrast, Brownian motion in three dimensions describes a sparse trajectory and it is difficult to hit a small target. This dependence on the dimensionality was originally investigated by Pólya showing that on a lattice a random walk in one and two dimensions is recurrent (i.e., the return probability to the original site is one), while it is not in three dimensions. For the latter case a random walker may escape completely from a once visited site [8, 9]. While we will consider the search problem in three dimensions below when discussing the facilitated diffusion model for gene regulation we here concentrate on the lower dimensional case.

Shlesinger and Klafter promoted the elegant idea that a non-Brownian random walk may to an appreciable extent avoid oversampling [10]. Such a non-local random walk model suggested by these authors is a Lévy flight, a random walk process with variable jump length [8, 11]: each jump length is drawn from a probability density function $\lambda(x)$ with characteristic function

$$\lambda(k) = \int_{-\infty}^{\infty} e^{ikx}\lambda(x)\,dx = \exp(-\sigma^\alpha |k|^\alpha) \sim 1 - \sigma^\alpha |k|^\alpha, \tag{1}$$

for $0 < \alpha \leqslant 2$. The asymptotic expansion is valid for the so-called diffusion limit $\sigma^\alpha |k|^\alpha \ll 1$. While in the limit $\alpha = 2$ the jump length distribution $\lambda(x)$ is Gaussian with variance $\langle x^2\rangle = 2\sigma^2$ and the resulting random walk converges to a Brownian walk [8], in the domain $0 < \alpha < 2$, $\lambda(x)$ becomes a symmetric Lévy stable law with asymptotic expansion

$$\lambda(x) \sim A_\alpha \frac{\sigma^\alpha}{|x|^{1+\alpha}} \tag{2}$$

such that its variance diverges. This scale freeness of the jump length distribution translates into the fact that the fractal dimension of the resulting trajectory equals the Lévy index: $d_f = \alpha$. Clearly, this offers the possibility of creating trajectories whose dimension is smaller than the embedding dimension, and thus reduces oversampling.

However the possibility of performing very long jumps, allowed by the long-tailed form of $\lambda(x)$ for $0 < \alpha < 2$, causes pronounced leapovers [12–14] across a point. Consider a

particle that starts its motion at point $x = 0$. After a number of steps it will cross the point $x = d$ for the first time. The leapover distance ℓ measures how much the particle overshoots the point $x = d$ in a single jump. These leapover lengths are distributed according to the long-tailed law [15]

$$\wp_l(\ell) = \frac{\sin(\pi\alpha/2)}{\pi} \frac{d^{\alpha/2}}{\ell^{\alpha/2}(d+\ell)}. \tag{3}$$

In the limit $\alpha \to 2$, $\wp_l(\ell)$ tends to zero if $\ell \neq 0$ and to infinity at $\ell = 0$ corresponding to the absence of leapovers in the Gaussian continuum limit. However, for $0 < \alpha < 2$ the leapover distribution follows an asymptotic power law with index $\alpha/2$, and is thus broader than the original jump length distribution, $\lambda(x)$, with index α. This is remarkable: even while λ for $1 < \alpha < 2$ still has a finite characteristic length $\langle|x|\rangle$, the mean leapover length diverges. This finding is related to the property of a Lévy flight that the first arrival probability at a point is strongly reduced, leading to the asymptotic time dependence [16]

$$\wp_{\rm fa}(t) \simeq \frac{d^{\alpha-1}}{t^{2-1/\alpha}}, \qquad 1 < \alpha < 2. \tag{4}$$

This is a much broader distribution than the first arrival of a Brownian walker with $\wp_{\rm fa}(t) \simeq t^{-3/2}$. For $0 < \alpha < 1$ the first arrival at an infinitely small point target becomes impossible. Thus while Lévy flights reduce oversampling, they also have a low probability to hit a small, point-like target.

A successful way out of this oversampling-versus-arrival probability at the target dilemma can be overcome by intermittent search models that combine different search mechanisms [17, 18]. Let us consider effectively one-dimensional search processes. This may be relevant for the search at interfaces (e.g., along riverbanks or at the forest–meadow border), in small channels or in long nerve cells. Here we consider the simplest case, the combination of two different mechanisms, these being fast directed relocation events with a velocity v where no detection of the target is possible, and local diffusional search for the target with diffusivity D. A sketch of the resulting process is shown in figure 1. The relocation events provide a fast escape from a local area scanned in the previous diffusion event, while the diffusion periods serve local exploration and, ultimately, detection. From this reasoning it is already clear that the span of a single diffusion period should be smaller than the typical distance travelled in a relocation event.

Mathematically we phrase this model as follows, already setting the stage for the formalism applied in the modelling of facilitated diffusion. We assume that the diffusive search phase switches over to the relocation phase with probability per time τ_1^{-1}. In this relocation phase the searcher moves ballistically with velocity v in a random direction, i.e., the searcher does not possess an orientational memory, as also assumed previously in [19]. This is, for instance, a reasonable model for the relocation of bacteria during a tumbling event, in absence of an external signal gradient [20, 21]. The time span of such a relocation event is drawn from the waiting time distribution $\psi(t)$, which we consider to be either exponential or Lévy stable, see below. For consistency with previous approaches we use a closed cell approach: the search is performed on an interval of length L with periodic boundary conditions, equal to regularly spaced targets with density $1/L$. The model can be formulated as an equation for the probability density $P(x,t)$ for the position x at time t of the searcher in the search phase

$$\frac{\partial P}{\partial t} = \frac{1}{\tau_1}\int_{-L/2}^{L/2} {\rm d}x' \int_0^\infty {\rm d}t' W(x-x', t-t') P(x', t') - \frac{1}{\tau_1} P(x,t) + D\frac{\partial^2 P}{\partial x^2} - \wp_{\rm fa}(t)\delta(x). \tag{5}$$

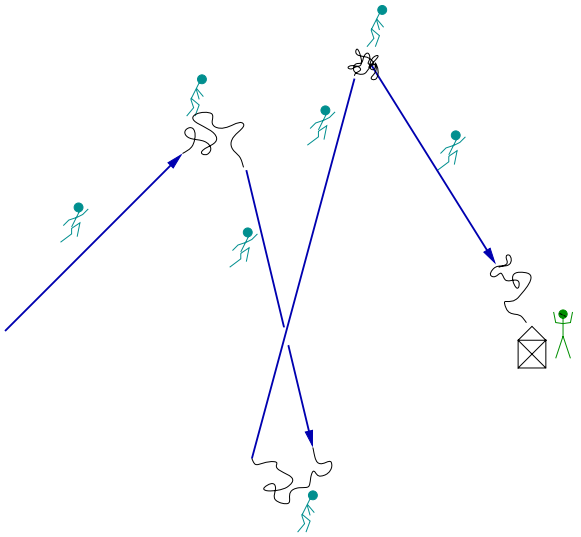

Figure 1. Simple model of intermittent search, in which directed relocations with velocity v follow local diffusion events with diffusivity D.

According to the last term on the right-hand side the searcher is removed when it arrives at the target, which is placed at $x = 0$. The probability density $\wp_{\rm fa}(t)$ thus is the first arrival time at the target, which is determined implicitly by the absorbing boundary condition $P(x = 0, t) = 0$. The term proportional to the diffusion constant D accounts for the local Brownian motion in the search phase. By $-\tau_1^{-1} P(x, t)$ the searcher is removed from location x with rate constant τ_1^{-1}. The searcher is then relocated according to the convolution integral whose kernel $W(x, t)$ is the joint probability density of making a relocation of length x during a time t. It is defined by

$$W(x, t) = \frac{\psi(t)}{2} \sum_{n=-\infty}^{\infty} \delta(|x + nL| - vt). \tag{6}$$

In this expression the δ-coupling enforces that the distance travelled in time t is vt. This spatiotemporal coupling is the simplest version of a Lévy walk [22], in which there exists a maximum distance that can be travelled in a certain time span, such that the variance of a Lévy walk is finite. This contrasts the Lévy flight with its infinite variance discussed above. Finally the sum over n guarantees that $W(x, t)$ is L-periodic in x. Due to the spatiotemporal coupling the probability density $\psi(t)$ is related to the spatial distribution of the relocations $\lambda(x)$ through

$$\psi(t) = 2v\lambda(vt). \tag{7}$$

We assume that the jump length distribution $\lambda(x)$ is symmetric around $x = 0$ (no orientational memory/no bias).

A typical quantity used to gauge the efficiency of a search process is the characteristic search time

$$\langle t \rangle = \int_0^\infty {\rm d}t \, t \wp_{\rm fa}(t). \tag{8}$$

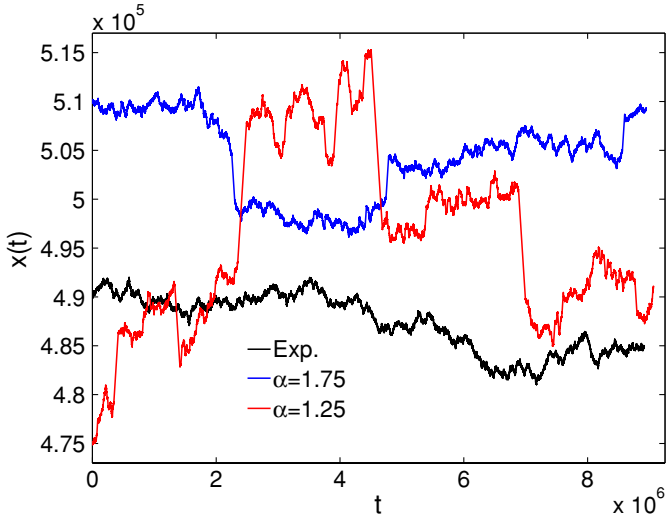

Figure 2. *x–t* diagram with exponential and Lévy relocations. As parameters we use $\tau_1 = 37, \tau_2 = 200, D = 1, v = 0.1$. As we focus on the trajectory here and not on the target location event we choose $L = \infty$.

In the derivation of the main results we assume that the initial distribution of the searcher is uniform, $P(x, t = 0) = 1/L$, since there is no *a priori* information on the target position. We distinguish two cases.

In a Lévy walk scenario for the relocations, we assume a symmetric Lévy distribution for the jump lengths, with characteristic function (1), corresponding to the long-tailed asymptotic behaviour (2). Here, however, we restrict the Lévy index to $1 < \alpha < 2$ such that the mean relocation time $\tau_2 = \int_0^\infty t\psi(t)\,\mathrm{d}t$ is finite. In figure 2 we show sample trajectories for the cases of exponential and Lévy distributed relocations. The Lévy case is clearly distinguished with its, occasional, long relocations.

We obtain the optimal time scales τ_1 and τ_2 by simultaneously demanding $\partial\langle t\rangle/\partial\tau_1 = 0$ and $\partial\langle t\rangle/\partial\tau_2 = 0$. It turns out that the τ_i scale like $L^{(\alpha-1)/(\alpha-1/2)}$ with the system size L, implying the scaling [23]

$$\langle t\rangle \simeq L^{(3\alpha-2)/(2\alpha-1)} \tag{9}$$

for the search time: for large L the most efficient search will occur for α close to 1. This is consistent with the different search model developed in [24]. Note however that the prefactor to the L-scaling diverges as $\alpha \to 1$, so the optimal choice of α will be somewhat larger than 1 for any finite L. This is demonstrated in figure 3.

In contrast, for exponentially distributed relocation periods with

$$\psi(t) = \tau_2^{-1}\,\mathrm{e}^{-t/\tau_2}, \tag{10}$$

we obtain the scaling $\tau_i \simeq L^{2/3}$, and $\langle t\rangle \simeq L^{4/3}$ for the search time, in agreement with reference[8] [19].

Comparison of the $L^{4/3}$ scaling for exponentially distributed relocations with the scaling $\langle t\rangle \simeq L^{(\alpha-1)/(\alpha-1/2)}$ for Lévy relocations with $1 < \alpha < 2$ proves that the latter are increasingly more efficient for decreasing target density (large L). For very rare targets the Cauchy strategy

[8] Note the typo in the expression for $\langle t\rangle$ in equation (5) of [19]: $\coth(1/[2\alpha L])$ should be $\coth(\alpha L/2)$.

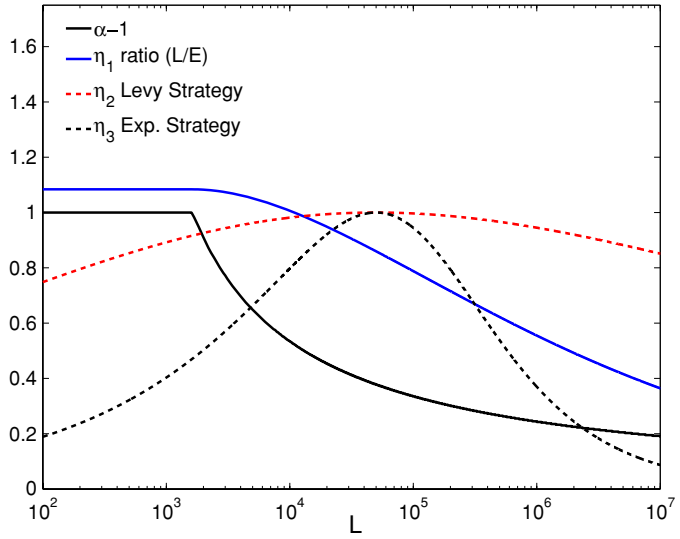

Figure 3. We show the optimal α for Lévy relocation strategy, and the ratio $\eta_1 \equiv \langle t\rangle^{\alpha}_{\rm opt}/\langle t\rangle_{\rm exp}$ of search times for optimal α in the Lévy scenario versus the exponential strategy, as a function of L (full lines). Moreover we depict the ratio $\eta_2 = \langle t\rangle_{L,\tau_i(L)} / \langle t\rangle_{L,\tau_i(L_0)}$ of optimal versus fixed τ_i search times as a function of L for the Lévy strategy with $\alpha = 1.25$ as well as the corresponding ratio η_3 for the exponential case; for both the fixed τ_i were optimized for $L_0 = 5 \times 10^4$ (dashed lines).

becomes the limiting most efficient strategy, pointing to the somewhat peculiar role of the Cauchy distribution: while for $1 < \alpha < 2$ the variance $\langle x^2\rangle$ diverges, at least the first absolute moment $\langle |x|\rangle$ remains finite. This last remaining scale vanishes at $\alpha = 1$. Given that also other search models recover that $\alpha = 1$ optimizes the search for rare targets this fact may turn out to be universal in some sense.

An additional advantage of Lévy strategies is the following. Due to the scaling $\tau_i \simeq L^{(\alpha-1)/(\alpha-1/2)}$ of the optimal time scales τ_i we see that for α close to unity the optimal strategy becomes independent of the target density. This means that it is increasingly less important for the searcher to have advance knowledge of the density of targets L^{-1} if it follows a small α Lévy strategy, since it can choose τ_i that are almost optimal over a broad range of densities, as illustrated in figure 3 by the dashed lines. One may therefore speculate whether for creatures without long-range memory Lévy search strategies may be evolutionary advantageous. We should note that these results are obtained for an effectively one-dimensional system. In higher dimension, and depending on additional boundary conditions, above effects may become less pronounced. However a large body of data obtained for a wide range of species show Lévy-type foraging patterns thus supporting claims that indeed the scale-free nature of Lévy relocation lengths is beneficial.

3. Gene regulation and the role of the DNA configuration

DNA encodes the genetic information for proteins and enzymes, divided into individual genes. According to the central dogma of molecular biology [2, 3, 5, 25] the code of a gene is read out by RNA polymerase and subsequently converted into proteins in biochemical facilities called ribosomes. At any time the cell has only part of its genes 'turned on' while others are silent,

depending on cell development or external signals. Proteins that are necessary throughout the cell's life, such as molecular motors, proteins defending the cell against intruding DNA from viruses, or RNA polymerases, are being produced constantly. Other genes are turned on when necessary. A classical example is the lactose metabolism in *E. coli*: in the absence of lactose the Lac repressor is on and prevents unnecessary production of the lactose enzyme needed to digest lactose. Also well studied is the λ switch in *E. coli* infected by phage λ: this genetic unit after infection by the bacteriovirus decides between a dormant state in which the viral DNA is silently replicated along with the host cell's, and the lytic state in which new viruses are produced and the host cell is eventually killed [4]. Molecularly, the regulation of such genes relies on the presence of certain DNA-binding proteins (transcription factors) that bind to a specific binding site close to the starting sequence of the related gene. The transcription factor promotes or inhibits binding of RNA polymerase and subsequent production of the associated proteins/enzymes. This strategy allows eukaryotic and prokaryotic cells as well as viruses to regulate the production (and therefore consumption of resources) according to the cell development, internal or external signals [2, 4]. To understand how these transcription factors locate and subsequently bind to their specific binding site has been actively researched for several decades, remarkably by Adam and Delbrück [26], Richter and Eigen [27], and in the seminal work by Berg and von Hippel [6, 28, 29].

The search performed by the transcription factors is purely stochastic. Let us first concentrate on understanding the search process *in vitro*, where so far most research has been performed under dilute solvent conditions. Accordingly we may view DNA as a flexible polymer that even in bacteria is several mm long, corresponding to several megabases of information. The naive assumption would be to consider the transcription factors' search for their binding site as simple three-dimensional diffusion with diffusivity D_{3d}, searching for a target of size b. The famed Smoluchowski result for this process can be derived from the steady state solution of the radially symmetric diffusion equation for the volume density n of transcription factors

$$0 = \frac{\partial n}{\partial t} = D_{3d}\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial n}{\partial r}\right), \tag{11}$$

with the boundary conditions of a fixed density far away, $n \to n_{bulk}$ as $r \to \infty$, and immediate absorption at the surface of the target, $n|_{r=b} = 0$. From the solution

$$n = n_{bulk}(1 - b/r), \tag{12}$$

the rate constant for the binding reaction can be obtained from the stationary binding flux j_{stat} as

$$k_{on}^S = \frac{j_{stat}}{n_{bulk}} = \frac{1}{n_{bulk}} 4\pi b^2 D_{3d}\left.\frac{\partial n}{\partial r}\right|_{r=b} = 4\pi D_{3d} b, \tag{13}$$

which is the Smoluchowski result [30]. Note that this on rate has physical dimension $[k_{on}^S] = \mathrm{cm}^3\ \mathrm{s}^{-1}$. For a typical diameter of a transcription factor of 5 nm by Stokes formula we obtain $D_{3d} \approx 10^2\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$, and the typical target site size of one base pair corresponds to $2b \approx 0.3$ nm. Consequently $k_{on}^S \approx 10^8(\mathrm{mol\,l}^{-1}\mathrm{s})^{-1}$ in biochemical units. Even though this number does not include the binding of the transcription factor itself it is much smaller than some measured on rates: for the Lac repressor, for instance, $k_{on} \approx 10^{10}(\mathrm{mol\,l}^{-1}\,\mathrm{s})^{-1}$ was found [31].

Why can the transcription factors be faster than the three-dimensional diffusion limit? The answer to this riddle is provided by the facilitated diffusion model, another example for an intermittent search process. The facilitated diffusion scheme was sublimated in the works of Berg and von Hippel which are based on the fact that the presence of the DNA chain

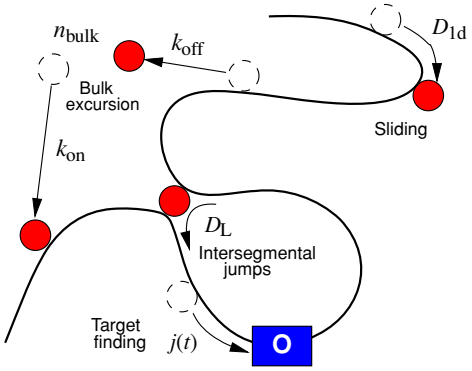

Figure 4. Facilitated diffusion model à la Berg and von Hippel [6, 28]: a DNA binding protein on its search for the specific target site switches between bulk (3D) diffusion, 1D diffusion along the DNA, and intersegmental jumps or transfers.

provides the topological grounds for different search mechanisms, see figure 4. Accordingly DNA-binding proteins in addition to three-dimensional diffusion intermittently also diffuse one-dimensionally along the DNA chain as experimentally proven [32–35]. This linear diffusion is mediated by non-specific binding, the binding affinity of transcription factors to non-target DNA, made possible by the heteropolymeric nature of DNA [36, 37]. In addition Berg and von Hippel also proposed the mechanism of intersegmental transfers during which the transcription factor non-specifically binds to two DNA sites, brought into contact by DNA looping, and randomly transfers to either site. This makes it possible to jump a long distance in the chemical coordinate measured along the DNA [6].

In our model for facilitated diffusion we formulate the generalized diffusion equation [38]

$$\frac{\partial n(x,t)}{\partial t} = \left(D_{1\text{d}}\frac{\partial^2}{\partial x^2} - k_{\text{off}}^{\text{ns}}\right) n(x,t) - j(t)\delta(x) + G(x,t) + k_{\text{off}}^{\text{ns}} \int_{-\infty}^{\infty} \text{d}x' \int_0^t \text{d}t' W_{\text{bulk}}(x-x', t-t') n(x', t'), \tag{14}$$

for the line density $n(x,t)$ of transcription factors on the DNA, measured along the chemical coordinate x along the DNA chain. Here, the first term on the right-hand side stands for the one-dimensional motion along the DNA with effective diffusivity $D_{1\text{d}}$. The second term is the off rate for non-specifically bound proteins unbinding from the DNA into the bulk. The third term, assuming that the specific binding site is placed at $x = 0$, is the flux of arriving transcription factors that are being removed by the incorporated δ sink. The belonging boundary condition is $n(x,t) = 0 \ @ \ x = 0$. The last term on the right is a transfer integral that relates the unbinding from the DNA at position x' at time t' with the rebinding at x and t. This transfer is mediated by the kernel W_{bulk} that will be considered below. Finally, $G(x,t)$ describes the virgin flux onto the DNA of transcription factors that have previously not been bound.

Similarly to the derivation of the Smoluchowski result, it is possible to derive the steady state solution for the binding flux $j_{\text{stat}} = k_{1\text{d}} n_{\text{eq}}^{\text{ns}}$ for the above model also [38]. Here $n_{\text{eq}}^{\text{ns}}$ is the one-dimensional density of proteins on the DNA at non-specific equilibrium and

$$k_{1\text{d}}^{-1} = \int_{-\infty}^{\infty} \frac{\text{d}q}{2\pi} \frac{1}{D_{1\text{d}}q^2 + k_{\text{off}}^{\text{ns}}(1 - \lambda_{\text{bulk}}(q))} \tag{15}$$

is a rate constant for the target search on the DNA. We have here also introduced $\lambda_{\rm bulk}(q)$, which is the Fourier transform of the distribution of relocation lengths through the bulk

$$\lambda_{\rm bulk}(q) = \int_0^\infty {\rm d}t \int_{-\infty}^\infty {\rm d}x\, {\rm e}^{{\rm i}qx} W_{\rm bulk}(x,t). \tag{16}$$

Again one can define a rate constant with respect to the bulk density of proteins away from the DNA: $k_{\rm on} = j_{\rm stat}/n_{\rm bulk}$.

3.1. The straight rod DNA configuration

Let us at first consider DNA in the configuration of a straight rod. This is of interest for two reasons, namely, for historical reasons as it corresponds to the situation solved by Berg and Ehrenberg [39]. Additionally, such a straight configuration can in fact be realized experimentally, for example, by optical tweezers techniques as discussed below.

In this case the transfer kernel $W_{\rm bulk}$ can be calculated exactly from the cylindrical diffusion equation, and expressed in terms of Bessel functions [38]. To proceed and evaluate the search equation (15) one needs to resort to numerical methods. However there exist two limiting cases that can be explicitly evaluated. These can be distinguished by the non-specific reaction rate of transcription factors with the DNA, $k_{\rm on}^{\rm ns}$, defined by the equilibrium condition $n_{\rm eq}^{\rm ns} k_{\rm off}^{\rm ns} = n_{\rm bulk} k_{\rm on}^{\rm ns}$. Here the dimensions are those of a rate for $\left[k_{\rm off}^{\rm ns}\right] = {\rm s}^{-1}$, a line density $\left[n_{\rm eq}^{\rm ns}\right] = {\rm cm}^{-1}$, a volume concentration $[n_{\rm bulk}] = {\rm cm}^{-3}$, and therefore the non-specific reaction rate has the dimension of diffusivity, $\left[k_{\rm on}^{\rm ns}\right] = {\rm cm}^2\,{\rm s}^{-1}$. We then have the limiting cases:

(a) The high diffusivity case $k_{\rm on}^{\rm ns} \ll D_{\rm 3d}$. In this case the rate of non-specific binding limits the binding of the proteins such that each protein after unbinding relocates by a long distance and becomes essentially uncorrelated from the previous binding spot. The search rate becomes

$$k_{\rm on} = 2 l_{\rm sl} k_{\rm on}^{\rm ns}. \tag{17}$$

Thus, a protein that binds within the sliding length $l_{\rm sl}$ of the target site is able to locate the target. This sliding length is defined as

$$l_{\rm sl} = \sqrt{\frac{D_{\rm 1d}}{k_{\rm off}^{\rm ns}}}. \tag{18}$$

In this limit the configuration of DNA does not come into play at all, as each bulk excursion is long and (almost) completely decorrelates two successive non-specific binding sites.

(b) In the opposite case $k_{\rm on}^{\rm ns} \gg D_{\rm 3d}$ particles rebind quickly after unbinding from the DNA. This leads to oversampling and thus to a reduced target search rate compared to equation (17). The final asymptotic result is

$$k_{\rm on} \sim 4\pi D_{\rm 3d} l_{\rm sl}^{\rm eff} \big/ \left[\ln\left(l_{\rm sl}^{\rm eff}/r_{\rm int}\right)\right]^{1/2}, \tag{19}$$

where $r_{\rm int}$ is the interaction distance between protein and DNA, and the effective sliding length

$$l_{\rm sl}^{\rm eff} = \sqrt{\frac{k_{\rm on}^{\rm ns}}{(2\pi D_{\rm 3d})}} l_{\rm sl} \tag{20}$$

can be interpreted as the regular sliding length renormalized by immediate rebinding events [28]. The result (19) is essentially equivalent to the Smoluchowski result, with the target size replaced by the effective sliding length. Sometimes this increase of the target size to $l_{\rm sl}$ is called the antenna effect.

Our model produces the same results for the straight rod configuration as the calculation in the original paper by Berg and Ehrenberg [39]. We now turn to the case of a coiled, random DNA configuration that can be incorporated in our model.

3.2. Coiled DNA configuration

To calculate the transfer kernel $W_{\rm bulk}$ explicitly for a fluctuating random chain would be a formidable task. We include a number of physically reasonable assumptions to obtain a good estimate for $W_{\rm bulk}$ that we show to be consistent with data obtained from single DNA experiments below.

3.2.1. Coil with persistence length. The persistence length ℓ_p of a polymer chain is the length over which tangent–tangent correlations decay when the polymer is subject to thermal fluctuations. In terms of the bending stiffness κ of the polymer, the persistence length is $\ell_p = 2\kappa/[k_BT]$ [40]. On length scales smaller than ℓ_p the polymer appears like a rigid rod while on longer scales it may be viewed as a flexible chain.

An approximate way to obtain the transfer kernel $W_{\rm bulk}$ for a random coil-like DNA configuration is to treat the DNA chain as an array of locally straight segments. Defining the density $l_{\rm DNA}$ of DNA (with dimension of length per volume) around a given segment, we assume that this density is dilute such that the typical distance $1/\sqrt{l_{\rm DNA}}$ between segments is much larger than the interaction radius $r_{\rm int}$. In analogy with Smoluchowski's derivation [30] we further assume that the probability that the protein has reacted with any segment is obtained from treating the interaction with each segment independently and then choosing among these the interaction for which binding happens first. To find the dynamics for the interaction of the protein with one given segment of DNA we consider a comparatively large volume V around the segment of length L.

Placing the protein at a uniformly random position in space relative to the DNA segment at $t = 0$ and denoting the probability that the protein has bound to this DNA before time t by $J_{\rm single}(t)$, then the probability that it has not bound to any of N pieces of DNA is (using the assumed independent interactions) $P_{\rm surv}^{\rm foreign}(t) = [1 - J_{\rm single}(t)]^N$. Taking the limit of $V, N, L \to \infty$ for fixed $l_{\rm DNA} = NL/V$ we get $P_{\rm surv}^{\rm foreign}(t) = \exp[-J_{\rm cap}(t)]$, where [38]

$$\frac{u^2 J_{\rm cap}(u)}{k_{\rm on}^{\rm ns} l_{\rm DNA}} = \left(1 + \frac{k_{\rm on}^{\rm ns} K_0(\sqrt{u/D_{\rm 3d}}\, r_{\rm int})}{2\pi\sqrt{u D_{\rm 3d}}\, r_{\rm int} K_1(\sqrt{u/D_{\rm 3d}}\, r_{\rm int})}\right)^{-1}, \tag{21}$$

and u is the Laplace variable corresponding to time t. $W_{\rm bulk}$ in the coiled conformation then becomes

$$W_{\rm bulk}(q,t) = W_{\rm bulk}^{\rm cyl}(q,t) P_{\rm surv}^{\rm foreign}(t) - \frac{{\rm d}P_{\rm surv}^{\rm foreign}(t)}{{\rm d}t}\delta_{q,0}\int_t^\infty W_{\rm bulk}^{\rm cyl}(q=0,t')\,{\rm d}t',$$

with q representing a Fourier transform with respect to x. The first term on the right-hand side stands for proteins that return to the original segment without being captured by foreign segments. Those captures are represented by the second term, where the factor $\delta_{q,0}$ is a result of the assumption that a capture by foreign DNA segments leads to a long relocation measured along the DNA contour. We call these captures by a foreign segment intersegmental jumps.

Extracting values of $k_{\rm on}$ numerically one finds that the significance of the intersegmental jumps is small when the sliding length $l_{\rm sl}^{\rm eff}$ is small compared with the typical distance between DNA segments: $k_{\rm on}$ will only be slightly larger than the corresponding value for a straight configuration of the DNA. However, the density of DNA segments $l_{\rm DNA}$ is in many situations enhanced compared with the density of a freely fluctuating semi-flexible polymer. This enhancement can, for instance, be mediated by supercoiling, attraction between segments induced by multivalent ions, or compaction by various kinds of proteins.

Without the assumption implicit in equation (22) that relocations due to intersegmental jumps are longer than the effective sliding length $l_{\rm sl}^{\rm eff}$ it would be much harder to obtain the

relevant parts of $W_{\rm bulk}$, since then the actual distribution of the intersegmental jump relocations would have to be known to evaluate the degree of oversampling. In the following we discuss a related limit involving intersegmental transfers.

3.2.2. Long flexible chain: Lévy flights. For very long chains we find an interesting result when we include intersegmental transfers but neglect correlations between the unbinding position to the bulk of a transcription factor and its point of rebinding to the DNA. This models transcription factors with small non-specific binding rate and small unbinding rate such that the sliding length in between unbindings and transfers is longer than the polymer's Kuhn length. With these approximations we rephrase our transport equation in the form [41]

$$\frac{\partial}{\partial t}n(x,t) = \left(D_{1d}\frac{\partial^2}{\partial x^2} + D_{\rm L}\frac{\partial^\alpha}{\partial|x|^\alpha} - k_{\rm off}\right)n(x,t) + k_{\rm on}n_{\rm bulk} - j(t)\delta(x), \tag{23}$$

where we combine the one-dimensional Brownian diffusion along the DNA chain, unbinding from the DNA with rate $k_{\rm off}$, and the rate of non-specific rebinding from the bulk is here labelled $k_{\rm on}$. For the latter we assume a constant bulk density which is only slightly perturbed by the binding and unbinding dynamics to and from the DNA chain. The last term again represents the target, the δ sink allowing us to calculate the first passage into the target. Finally, the intersegmental transfers are represented by the second term on the right-hand side, which is a fractional derivative in space defined through its Fourier transform, $\int_{-\infty}^{\infty} {\rm e}^{{\rm i}kx}\frac{\partial^\alpha}{\partial|x|^\alpha}n(x,t)\,{\rm d}x = -|k|^\alpha n(k,t)$, where we denote the Fourier transform by explicit dependence on the wave number k. This term corresponds to a jump length distribution of the form $\lambda(x) \simeq \sigma^\alpha|x|^{-1-\alpha}$ [11]. We assume that these Lévy jumps occur with a rate τ such that the effective diffusivity $D_{\rm L}$ of dimension cm$^\alpha$ s^{-1} can be obtained. The jump length distribution $\lambda(x)$ stems from the fact that in polymer physics the length ℓ stored in a random loop of a flexible, long polymer scales like [42]

$$p(\ell) \simeq \ell^{-c}, \tag{24}$$

where c is the critical exponent for the contact probability. For a chain in a good, dilute solvent $c \approx 1.2$, while for a Gaussian chain (θ solvent) $c = 2.2$. As a transcription factor can use this contact between two DNA segments that are remote measured along the DNA but close by in the embedding space to jump backwards and forwards along the chain this gives rise to the jump length distribution $\lambda(x)$ of the above power-law form, where we identify $\alpha = c - 1$. For all flexible polymers this is indeed a Lévy flight with diverging variance.

In this model we observe that the Lévy flight search mechanism aids the search efficiency. Optimizing the ratio of time the transcription factor spends on the DNA versus in the bulk is changed from one (equal amount of time spent in bulk and on DNA for minimum search time) when $D_{\rm L} = 0$ to $(\alpha - 1)$ for good solvent conditions and sufficiently large $D_{\rm L}$. Equivalently, under bad solvent conditions leading to Gaussian chain behaviour, for larger $D_{\rm L}$ the most efficient search is performed by transcription factors that never unbind from the DNA. Although this model is an approximation it shows that when the DNA configuration is taken into account bulk diffusion becomes less relevant. This is good news when trying to understand gene regulation in living cells where volume diffusion appears to be affected by molecular crowding, see below.

3.3. On rates in single DNA experiments with variable DNA coiling

The influence of the DNA configuration on the search rate of DNA binding proteins can indeed be probed experimentally, as reported recently [43]. There, a dual optical tweezers setup was

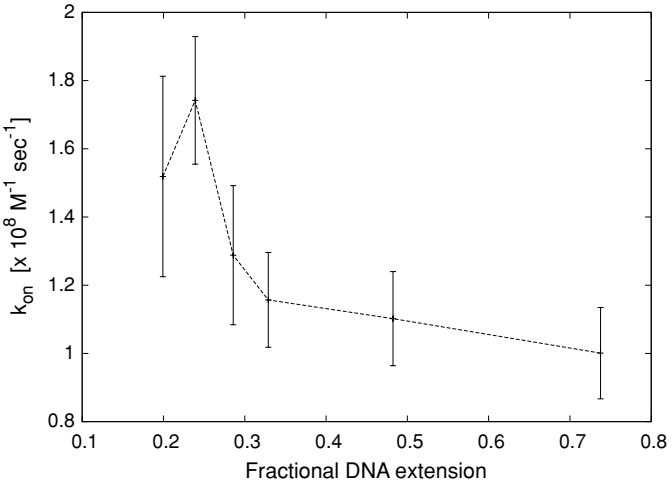

Figure 5. Target search rate in the EcoRV system as a function of the fractional DNA extension [43]. Compared to the base rate at almost fully stretched DNA there appears a maximum at around 0.24, corresponding to the natural gyration radius of the unstretched chain. Salt concentration: 100 mM NaCl and 5 mM Mg.

used to hold a single DNA molecule of length $L = 6538$ base pairs (approximately 2.2 μm) in place. This DNA contained one specific binding site for EcoRV restriction enzymes that were in solution. After binding to this site an EcoRV will cut the DNA. By the two tweezers the DNA was held at a variable equilibrium extension. Every second the DNA was quickly stretched to probe whether the DNA was still intact. Starting from an almost completely stretched DNA in a series of measurements each cycle the chain was further relaxed. For each equilibrium distance of the two DNA end points the associated cleavage rate was measured. As the cleavage as such is shorter than the target search one may therefore infer the influence of the DNA conformation on the search rate. The result is plotted in figure 5.

Interestingly there occurs a pronounced maximum at a fractional extension of roughly 0.24. This corresponds to the maximally random configuration when the DNA size is characterized by the gyration radius of the free DNA chain. The relative increase compared to the almost fully stretched configuration is significant, about 1.7. Similar measurements were performed at different NaCl concentrations. Remarkably the pronounced maximum seen in figure 5 is decreased significantly at different NaCl concentration. For a quantitative modelling it is not trivial to include the salt dependence, as many model parameters become affected. For low NaCl concentration the experiment can be described in terms of a local DNA density obtained from the worm like chain model for DNA with persistence length $\ell_p \approx 50$ nm. At higher NaCl concentrations and simultaneous presence of bivalent Mg^{2+} counterions (in our experiments 5 mM Mg^{2+} was used) attractive interactions between DNA segments arise [44, 45]. With reasonable values for this density the observed search rates can be explained, for details, see [43]. Since EcoRV has only one DNA binding site and therefore cannot perform intersegmental transfers this experiment is direct proof for the existence of intersegmental jumps. We note that the increase of the search rate effected by intersegmental jumps should be further pronounced when DNA is packaged, for instance, in a cell. A similar increase by a factor of 3 to 4 was in fact observed in supercoiled versus relaxed plasmids [35].

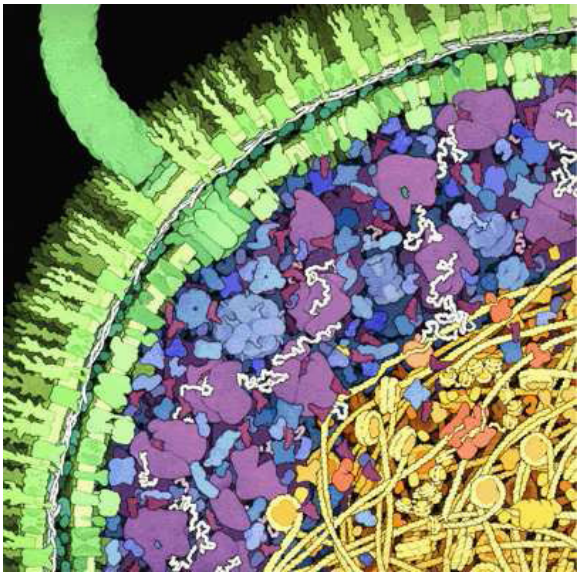

Figure 6. View of the degree of molecular crowding in a bacteria cell. At bottom right one can see the compactified DNA, in the cytoplasm there are ribosomes, transcription factors and messenger RNA (white). Courtesy David Goodsell, the Scripps Research Institute.

4. In a living cell things are different

Inside living cells the density of larger biopolymers is surprisingly high, taking up approximately 40% of the volume. This *molecular crowding* is beautifully captured in David Goodsell's drawing reproduced in figure 6. The significance of molecular crowding to all sorts of biochemical processes such as enzyme action was already proclaimed by Arthur Kornberg in his Ten Commandments commentary [46]. Even for moderately large transcription factors (above 100 kD molecular weight and some 5 nm diameter) recent literature suggests pronounced effects on the diffusion properties. We briefly comment on this effect in this section.

4.1. Subdiffusion of transcription factors?

There exists a number of experimental claims that diffusion of molecules in dense environments deviates from the archetypical linear time dependence $\langle r^2(t)\rangle \simeq Kt$ of the mean-squared displacement. Instead anomalous diffusion of the type [11, 47–49]

$$\langle r^2(t)\rangle \simeq K_\gamma t^\gamma \tag{25}$$

with $0 < \gamma < 1$ is reported. In particular, by single particle tracking methods it was shown that adeno-associated viruses of radius $\approx$ 15 nm in a cell may perform subdiffusion with $\alpha = 0.5 \ldots 0.9$ [50]; fluorescently labelled messenger RNA chains of length 3000 bases and diameter of some 50 nm subdiffuse with $\alpha \approx 0.75$ [51]; lipid granules of typical size of a few hundred nm follow equation (25) with $\alpha \approx 0.75 \ldots 0.85$ [52–54]. By fluorescence correlation spectroscopy subdiffusion was observed for membrane protein motion ($\alpha \approx 0.5 \ldots 0.8$) [55] and dextrane polymers of various lengths in living cells and reconstituted crowded environments ($\alpha \approx 0.5 \ldots 1$) [56, 57]. In these experiments the exact physical nature of the subdiffusion is yet unknown, as well as the time scale over which the subdiffusion persists.

The nature of subdiffusion is known more precisely from single tracking of submicron plastic beads in a reconstituted actin network of typical mesh size ξ: the subdiffusion was shown to be associated with a power law waiting time distribution $\psi(\tau) \simeq \tau^{-1-\gamma}$[9] whose index γ varies between close to zero for $a/\xi \approx 1$ and larger up to one for $a/\xi \approx 0.3$ where a is the bead size [58]. For larger particles such as lipid granules the subdiffusion may therefore be connected to the viscoelasticity of the medium, similar to the *in vitro* experiments of [58]. For smaller particles the situation is less clear and for the moment remains open. Either way three-dimensional diffusion in a living cell will be significantly reduced with respect to diffusion at dilute solvent conditions. Moreover, the configuration dynamics of the DNA chain will be strongly affected.

4.2. Single particle trajectories: pitfalls in data analysis

In a single particle tracking experiment one samples individual particle trajectories, typically in the form of x–y time series. Characteristic quantities are then calculated as time averages. For instance, the time-averaged mean-squared displacement is obtained as

$$\overline{\delta^2}(\Delta, T) = \frac{1}{T-\Delta}\int_0^{T-\Delta} [x(t'+\Delta) - x(t')]^2 \, \mathrm{d}t', \tag{26}$$

where T is now the overall measurement time. Another, more conventional, way to calculate the mean-squared displacement is the ensemble average, $\langle x^2(t)\rangle = \int x^2 P(x,t)\,\mathrm{d}x$. For normal diffusion $\overline{\delta^2}(\Delta, T) = 2K\Delta$ such that Δ takes on the role of time of the ensemble average, and both are completely equivalent. Note that the time average is independent of the measurement time, T. The same is true for fractional Brownian motion [59] for which now $\overline{\delta^2}(\Delta, T) = 2K_\gamma \Delta^\gamma$ where now $0 < \gamma < 2$, with γ larger or smaller than one depending on whether the fractional Brownian motion is persistent or antipersistent [60].

However, for a random walk process in which the waiting times between successive jumps are distributed in the form of a power-law distribution $\psi(\tau) \simeq \tau^{-1-\gamma}$ the emerging subdiffusion process is strongly non-local in time and displays ageing. The time-averaged mean-squared displacement now explicitly depends on T, and the ensemble average of $\overline{\delta^2}(\Delta, T)$ turns out to be [61–63]

$$\langle\overline{\delta^2}(\Delta, T)\rangle \sim \frac{2K_\gamma}{\Gamma(1+\gamma)}\frac{\Delta}{T^{1-\gamma}}, \tag{27}$$

in the limit $\Delta \ll t$. In this case the Δ-dependence is the same as for normal diffusion. Concentrating solely on the Δ-dependence may therefore lead to the wrong conclusion to measure normal diffusion in the data analysis of measured single trajectories. The anomaly represented by the scaling exponent γ enters solely into the dependence on the overall measurement time T: for increasing system evolution the effective diffusion exponent $K_\gamma/T^{1-\gamma}$ decays in time, reflecting the scale-free nature of the waiting time distribution ψ: each time a waiting time is drawn from ψ the result might be even larger than before. The behaviour (27) is related to the fact that for such systems the long time average (and therefore the ensemble average of the long time average) no longer equals the ensemble average, often referred to as weak ergodicity breaking [64, 65]. In a finite system the linear dependence on Δ is broken and the resulting turnover appears like a power law. Simulations of this system interestingly appear very similar to actually observed experimental data, see the discussion in [62].

[9] Waiting times of this form directly lead to the anomalous scaling (25) [11].

The time-averaged mean-squared displacements reported in [51] show a very pronounced scatter in the anomalous diffusion coefficient K_γ. This would be consistent with results for the distribution of the diffusivity K_γ found in [62]. Recent results on single trajectory records of lipid granule subdiffusion are reported to be independent of the location in the cell. If therefore the subdiffusion is of dynamic origin the strong scatter of the diffusivities may point towards an ageing system with power-law form of ψ. For fractional Brownian motion the scatter according to preliminary simulations results appears much less pronounced. Additional data will be necessary to find out more about the subdiffusion of larger biomolecules in the cell.

5. Discussion

What is optimal search? Like all optimization problems this question has several answers. For instance, do we want to find something quickly, how much energy are we willing to spend on the search (stochastic versus directed search)? How sensitive should the system be to environmental parameters? How many agents are searching in parallel? How much information do we have about the target? In general different systems will have different answers. For instance, passive proteins searching for their specific binding site are solely aided by the mechanisms of facilitated diffusion whereas a spider monkey in the Yucatan peninsula [66] in his mind essentially has his whole territory mapped and only needs to scan potential locations for ripe fruit. Currently search processes are receiving a lot of interest from physicists, as demonstrated in this special issue. The consequences of search or motion patterns can be tremendous: transcription factors that were not able to bind non-specifically could not find their target site efficiently enough. Or, without the high connectivity of modern human travel patterns diseases would not spread that rapidly [67].

Generally speaking search optimization requires the competition between two mechanisms: local search in order to spot the target, and decorrelation to avoid oversampling in the sense that already searched sites are not revisited continuously. As discussed here a single search mechanism usually does not provide this necessary duality. In this paper we mainly address the issue of intermittent search. While an agent that can move actively and combines directed relocations with local (diffusive) search will profit from long-tailed distributions of the relocation length in terms of search time and weak dependence on changing system parameters the picture is different for purely passive agents such as transcription factors searching for their specific binding site on a DNA chain. This search is pretty efficient given that some transcription factors occur at extremely small concentrations, down to a few nanomolar, and have to search megabases of DNA. Note that in a typical bacteria cell 1 nm corresponds to having one molecule per cell. While for a straight cylindrical DNA configuration Berg and Ehrenberg provided a mathematical understanding, we here discussed a new way to include correlations due to quick rebinding events of transcription factors by considering the binding to independent, locally straight DNA segments of some local density. It was shown by comparison to single DNA experiments that this model is able to quantitatively explain the observed search rates as a function of the variable local DNA density, imposed upon the system by the dual tweezers setup. While the assumption of a constant DNA density is not true for a large polymer whose fractal dimension is $1/\nu \leqslant 3$ we may argue that it is important to only consider correlations if the rebinding occurs quickly after the unbinding. Otherwise the transcription factor spends sufficient time in the three-dimensional diffusion mode that it essentially completely decorrelates, and can be captured by the zeroth Fourier mode [38].

In a living cell the situation is much less clear. Only a few models for the search of transcription factor are available. At the moment it is even not clear whether the subdiffusion of transcription factors is relevant and, for how long it might persist. It appears reasonable,

however, to assume that three-dimensional diffusion is considerably less efficient. Thus other search mechanisms will become more important. As discussed here the configuration of the DNA chain will significantly enhance the search efficiency. In addition locality of the regulation pathway will become very important for the search process. Remarkably such locality is indeed supported by recent bioinformatics research finding colocalization of gene regulation. It may be speculated whether the arrangement of the genome in a biological cell is optimized such that vital genetic units would be autoregulatory, or regulated by proteins encoded by colocalized genes. Thus reduced three-dimensional mobility would possibly allow the cell to operate genetic units efficiently at low concentrations of transcription factors.

Assuming that the motion of transcription factors under molecular crowding conditions indeed is subdiffusive we presented one possible stochastic model, the continuous time random walk subdiffusion with a waiting time distribution of the inverse power-law form $\psi(\tau) \simeq \tau^{-1-\gamma}$ $(0 < \gamma < 1)$ such that the characteristic waiting time $\langle\tau\rangle$ diverges. The consequences of this behaviour are connected to the ageing phenomenon. Namely we discussed weak ergodicity breaking: one of its consequences is that, while some transcription factors would leave the DNA and not make it back to the DNA for very long times, others would not manage to move far away from their previous binding position. This slow decorrelation from the initial position is typical for ageing systems, and might indeed support low global concentrations of transcription factors while having enhanced concentrations around their production sites close to their genes. If the subdiffusion is indeed of this type this would have intriguing consequences for the understanding of single molecule trajectories in a crowded system, as we discussed.

Another argument in favour of subdiffusion was brought forth by Guigas and Weiss [68]. If the subdiffusion were of the type of fractional Brownian motion then the fractal dimension of the sample path would become $2/\gamma$. For values of the anomalous diffusion exponent $\gamma \leqslant 2/3$ the trajectories of such a particle would become volume filling, providing a much higher encounter probability of the searcher with its target. While this scenario is appealing the strong scatter observed in the experiments of Golding and Cox [51] would rather agree with a continuous time random walk approach. To be able to decide on the actual diffusive behaviour in living cells will require additional experiments. Either way the consequences for our understanding of *in vivo* gene regulation will be interesting.

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