

Facilitated diffusion framework for transcription factor search with conformational changes

This content has been downloaded from IOPscience. Please scroll down to see the full text.

2015 Phys. Biol. 12 046012

(<http://iopscience.iop.org/1478-3975/12/4/046012>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

This content was downloaded by: pubad

IP Address: 89.202.245.164

This content was downloaded on 28/07/2015 at 13:58

Please note that [terms and conditions apply](#).

Physical Biology



PAPER

Facilitated diffusion framework for transcription factor search with conformational changes

Jérôme Cartailier¹ and Jürgen Reingruber^{1,2,3}¹ École Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France² INSERM U1024, Paris, France³ Author to whom any correspondence should be addressed.E-mail: reingrub@ens.fr**Keywords:** facilitated diffusion, mean first passage time, gene regulation, mathematical modeling, transcription factorSupplementary material for this article is available [online](#)RECEIVED
8 April 2015REVISED
5 June 2015ACCEPTED FOR PUBLICATION
26 June 2015PUBLISHED
22 July 2015**Abstract**

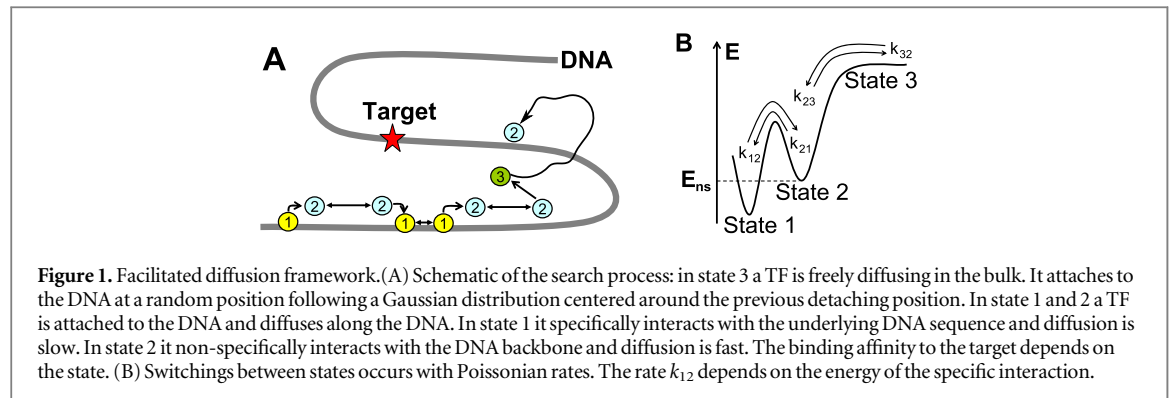
Cellular responses often require the fast activation or repression of specific genes, which depends on transcription factors (TFs) that have to quickly find the promoters of these genes within a large genome. TFs search for their DNA promoter target by alternating between bulk diffusion and sliding along the DNA, a mechanism known as facilitated diffusion. We study a facilitated diffusion framework with switching between three search modes: a bulk mode and two sliding modes triggered by conformational changes between two protein conformations. In one conformation (search mode) the TF interacts unspecifically with the DNA backbone resulting in fast sliding. In the other conformation (recognition mode) it interacts specifically and strongly with DNA base pairs leading to slow displacement. From the bulk, a TF associates with the DNA at a random position that is correlated with the previous dissociation point, which implicitly is a function of the DNA structure. The target affinity depends on the conformation. We derive exact expressions for the mean first passage time (MFPT) to bind to the promoter and the conditional probability to bind before detaching when arriving at the promoter site. We systematically explore the parameter space and compare various search scenarios. We compare our results with experimental data for the dimeric Lac repressor search in *E. coli* bacteria. We find that a coiled DNA conformation is absolutely necessary for a fast MFPT. With frequent spontaneous conformational changes, a fast search time is achieved even when a TF becomes immobilized in the recognition state due to the specific bindings. We find a MFPT compatible with experimental data in presence of a specific TF-DNA interaction energy that has a Gaussian distribution with a large variance.

Introduction

Transcription factors (TFs) regulate gene activation by binding to DNA promoter sites. To enable a fast cellular response that relies on the activation or repression of specific genes, TFs perform a facilitated diffusion search where they alternate between three dimensional (3D) diffusion in the bulk and 1D diffusion (sliding) along the DNA (for reviews see [1–6]). Initially, facilitated diffusion was introduced to explain the experimental finding that the *in vitro* association rate of the Lac-I repressor with its promoter sites placed on λ -phage DNA was around 100 times larger than the Smoluchowski limit $\sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for a

3D diffusion process [7]. Theoretical considerations showed that a search that alternates between 3D diffusion and 1D sliding can have a higher association rate compared to a pure 3D search [8–10]. With dilute DNA the search time is dominated by the 3D excursions between subsequent DNA binding events, and sliding increases the association rate by enlarging the effective target size (antenna effect). Later on single molecule techniques provided a direct experimental proof of the facilitated diffusion mechanism [11–14].

It has also soon been realized [9, 15] that frequent bindings to the DNA are problematic because sliding along the DNA is slow due to strong TF-DNA interactions [13, 14]. In a dense DNA environment with



frequent bindings to the DNA and slow 1D diffusion, the antenna effect becomes negligible and facilitated diffusion is slower compared to a pure 3D search. For example, in *E. coli* with a volume $|V| \sim 1 \mu\text{m}^3$, the measured search time of the Lac repressor for its promoter site is $\tau \sim 350 \text{ s}$ [11, 13]. This corresponds to an association rate $k_a = N_{Av} V / \tau \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$, much lower than the Smoluchowski limit. If a TF could specifically bind only to its promoter site and bounce off from the rest of the DNA, the search time would be extremely fast around $\sim V / (4\pi DR) \sim 5 \text{ s}$. However, because a TF cannot already recognize its target from the bulk, frequent DNA associations are essential. Thus, the question arises: How is a fast search possible within a large genome despite of facilitated diffusion?

When a TF is bound to the DNA and interacts with the underlying base pairs (bps), the diffusion coefficient for sliding decays exponentially with the variance of the binding energy distribution [16, 17]. With a simple facilitated diffusion model that comprises sliding along the DNA and uniform redistributions in 3D, one finds that a search time of the order of minutes is only compatible with a variance $\lesssim 1.5 k_B T$ [18]. In contrast, binding energy estimates for the *Cro* and *PurR* TF reveal a much larger variance around 5–6 $k_B T$ [18, 19]. This indicates that a simple diffusion process is not sufficient to explain the search dynamics when a TF is bound to the DNA. It has been proposed that a TF switches between two protein conformations with different binding affinities to the DNA [15, 18]. In the search conformation, a TF interacts only non-specifically with the DNA backbone leading to a smooth energy profile and fast diffusion. In the recognition conformation, a TF interacts specifically with the underlying DNA sequence resulting in a rough energy landscape and slow diffusion. Conformational changes of the TF protein are indeed supported by experimental observations [12, 20–24].

In this work we investigate a general framework for a facilitated diffusion search with conformational changes. We analytically derive the mean first passage time (MFPT) to bind to the target and the conditional probability to bind before dissociation when a TF arrives at the target site. We further compute the ratio

of the time spent in the bulk compared to attached to the DNA, the apparent diffusion constant for sliding along the DNA, and the average sliding distance before detaching. We consider a search process with Poissonian switchings between three states (figure 1). State 1 and 2 (recognition and search mode) correspond to two different protein conformations with conformation dependent TF-DNA interactions. Therefore the diffusion coefficients for sliding along the DNA and the target affinity depend on the conformation. In state 3, a TF is diffusing in the bulk and it associates with the DNA at a random position following a Gaussian distribution centered around the previous dissociation point. By modifying the target affinity in state 2 we evaluate the impact of induced switchings at the target site and the effect that a TF misses the target when arriving at the target site. By varying the correlation distance between dissociation and association point we estimate how the DNA conformation and coiling affect the MFPT. With our analytic expressions we can precisely evaluate the whole parameter space. We analyze various search scenarios within the same framework, which is important to accurately compare results. Other approaches partly rely on MFPT analysis, kinetic theory, thermodynamic equilibrium considerations, scaling arguments and other approximations, which complicates a comparison of results obtained with different methods and approximations [19, 25–30]. A clean MFPT analysis for a 3 states switching model with maximal target affinity in state 1 and no affinity in state 2 has been performed in [31]. Compared to [32], the authors additionally consider 3D excursions using a closed-cell approach as described in [9]. However, due to the difficulty to compute the 3D kernel, only the asymptotic limits corresponding to uniform redistributions and a rod-like DNA are discussed. The first passage time distribution for a switching process between two 1D states has been studied in [33].

Model

Model description and MFPT analysis

We start by presenting the mathematical framework and the MFPT analysis. We postpone the description

of the biological motivation to the results part. We consider a search that switches between 3 states with Poissonian switching rates k_{ij} (figure 1). In state 1 and 2 a TF is attached to the DNA of length $2L$ and slides with state dependent diffusion constants D_1 and D_2 . To simplify the analysis we consider that the target is located at the center. An off-centered target results in a higher MFPT up to maximally a factor of 4 if the target is located at the periphery (assuming that the MFPT scales $\sim L^2$) [34, 35]. When a TF reaches the target it binds with state dependent affinities χ_1 and χ_2 . In state 1 a TF switches to state 2 with rate k_{12} . In state 2, in addition to switching to state 1 with rate k_{21} , a TF can also dissociate with rate k_{23} and switch to state 3 where it diffuses in the bulk. From state 3 it associates with the DNA with the rate k_{32} at a random position drawn from a Gaussian distribution with variance σ_3^2 centered around the previous dissociation point.

Because of the Gaussian attaching distribution, we model state 3 as an 1D diffusion process along the DNA with an effective diffusion constant $D_3 = \frac{\sigma_3^2 k_{32}}{2}$ and no target affinity ($\chi_3 = 0$). Thus, we finally arrive at a framework with switchings between three 1D states. The backward Fokker–Planck equation for the probability $p(x, t, n|y, m)$ to find the TF at time t in state n at position x , conditioned that it started at $t = 0$ in state m at position y , is [36, 37]

$$\begin{aligned} \partial_t p(x, t, n|y, m) = & D_m \partial_x^2 p(x, t, n|y, m) \\ & - 2\chi_m p(x, t, n|y, m) \delta(y) \\ & - \sum_{i=1}^3 k_{mi} p(x, t, n|y, m) \\ & - p(x, t, n|y, i), \end{aligned} \quad (1)$$

with reflecting boundary conditions at $y = \pm L$. The mean sojourn time spent in state n is

$$\tau_{n,m}(y) = \int_0^\infty dt \int_{-L}^L dx p(x, t, n|y, m). \quad (2)$$

From equation 1 we find that the $\tau_{n,m}(y)$ satisfy the system of equations

$$\begin{aligned} D_m \tau_{n,m}''(y) - \sum_{i=1}^3 k_{m+i} \delta_{mi} - k_{mi} \tau_{n,i}(y) \\ - 2\chi_m \delta(y) \tau_{n,m}(y) = -\delta_{nm} \end{aligned} \quad (3)$$

with $k_{m+} = \sum_{j=1}^3 k_{mj}$. In the supplementary information (SI) we exactly solve equation 3 and derive analytic expressions for the $\tau_{n,m}(y)$. The MFPT when initially in state m at position y is $\tau_m(y) = \sum_{n=1}^3 \tau_{n,m}(y)$. We focus on the MFPT with uniform initial distribution, $\bar{\tau}_m = \frac{1}{2L} \int_{-L}^L \tau_m(y) dy$. Because switchings between states occur fast compared to the overall search time, the dependency of $\bar{\tau}_m$ on m is negligible. Furthermore, the mean sojourn times $\bar{\tau}_{i,m}$ approximately satisfy the scaling relations (see SI equation 40) $\bar{\tau}_{1,m} : \bar{\tau}_{2,m} : \bar{\tau}_{3,m} = 1 : \frac{k_{21}}{k_{12}} : \frac{k_{32}}{k_{23}}$. Hence, the MFPT with uniform initial distribution is well approximated by

$$\begin{aligned} \bar{\tau} & \approx \bar{\tau}_{1,1} \left(1 + \frac{k_{12}}{k_{21}} + \frac{k_{12}k_{23}}{k_{21}k_{32}} \right) \\ & = N_{12} \left(\frac{1}{k_{12}} + \frac{1}{k_{21}} + \frac{k_{23}}{k_{21}k_{32}} \right), \end{aligned} \quad (4)$$

where $N_{12} = \bar{\tau}_{1,1}k_{12}$ is the average number of switchings between states 1 and 2.

To reveal the scaling of the MFPT as a function of the DNA length L , we introduce the scaled length $\hat{L} = \frac{L}{L_0}$ defined with the reference length $L_0 = 1\text{bp}$. To simplify the discussion, we focus on a scenario with maximal affinity in state 1, $\chi_1 = \infty$, in which case the search is over when the TF encounters the target in state 1 (the analysis in the SI is performed with a general χ_1). For a long DNA ($\hat{L}^2 \mu_i \gg 1$) we compute in the SI

$$N_{12} = \hat{L} l_{12} \left(\frac{c_{1,1}}{\mu_1 \sqrt{\mu_1}} + \frac{c_{1,2}}{\mu_2 \sqrt{\mu_2}} \right) + \hat{L}^2 \frac{l_{12} l_{21} l_{32}}{3\beta} \quad (5)$$

with the following dimensionless parameters:

$$l_{ij} = \frac{L_0^2 k_{ij}}{D_i}, \quad c_{1,1} = l_{21} l_{32} \left(\frac{a_1 d}{e} + b_1 \right)$$

$$c_{1,2} = l_{21} l_{32} \left(\frac{a_2 d}{e} + b_2 \right)$$

$$\alpha = l_{12} + l_{21} + l_{23} + l_{32},$$

$$\beta = l_{21} l_{32} + l_{12} (l_{23} + l_{32}),$$

$$\mu_{1/2} = \frac{1}{2} \left(\alpha \pm \sqrt{\alpha^2 - 4\beta} \right)$$

$$a_1 = \frac{\mu_1 (l_{12} + l_{21} - \mu_2)}{l_{12} (\mu_1 - \mu_2)},$$

$$a_2 = \frac{\mu_2 (l_{12} + l_{21} - \mu_1)}{l_{12} (\mu_2 - \mu_1)},$$

$$b_1 = \frac{l_{32} - \mu_1}{l_{32} (\mu_1 - \mu_2)}, \quad b_2 = \frac{l_{32} - \mu_2}{l_{32} (\mu_2 - \mu_1)},$$

$$\kappa_2 = \frac{L_0 \chi_2}{D_2}, \quad d = \frac{1}{l_{32} \kappa_2} - \left(\frac{b_1}{\sqrt{\mu_1}} + \frac{b_2}{\sqrt{\mu_2}} \right),$$

$$e = \frac{a_1}{\sqrt{\mu_1}} + \frac{a_2}{\sqrt{\mu_2}} + \frac{l_{21}}{l_{12} \kappa_2}.$$

Search with uniform redistribution in state 3 and no target affinity in state 2

With uniform redistributions ($\sigma_3 = \infty$) we have $l_{32} = 2L_0^2/\sigma_3^2 = 0$. With $\kappa_2 = 0$ and $l_{32} = 0$, equation 5 simplifies to

$$N_{12} = \hat{L} l_{12} \left(\frac{l_{12} - \mu_2}{\mu_1 - \mu_2} \frac{1}{\sqrt{\mu_1}} + \frac{l_{12} - \mu_1}{\mu_2 - \mu_1} \frac{1}{\sqrt{\mu_2}} \right), \quad (6)$$

in agreement with [32]. We note that the term $\sim L^2$ in equation 5 vanished and we now have $N_{12} \sim L$, which leads to a faster search for large L . As stated before, equation 6 is valid for $\hat{L}^2 \mu_i \gg 1$ ($i = 1, 2$) and therefore cannot be applied for $k_{23} \rightarrow 0$. For $k_{23} \rightarrow 0$ one eigenvalue, say μ_1 , vanishes and the condition

$\hat{L}^2 \mu_1 \gg 1$ is violated. For example, with a fixed L and $k_{23} \rightarrow 0$ the TF remains bound to the DNA. In this case we expect that the MFPT scales $\sim L^2$ and not $\sim L$, which is indeed the case, as can be shown by a refined analysis. However, for any fixed value $k_{23} > 0$, by increasing L , N_{12} eventually scales $\sim L$ due to the uniform redistributions.

Optimal switching scenario in state 2

When the properties of state 1 and state 3 are fixed (and D_2 is fixed), we compute the optimal switching rates k_{21} and k_{23} that minimize the MFPT. We introduce the parameters

$$\sigma_1^2 = \frac{2D_1}{k_{12}}, \quad \sigma_2^2 = \frac{2D_2}{k_{21} + k_{23}}, \quad q = \frac{k_{23}}{k_{21} + k_{23}}, \quad \zeta = \frac{\sigma_1^2}{\sigma_2^2}. \quad (7)$$

σ_1^2 is the mean square displacement in state 1, σ_2^2 is the mean square displacement in state 2 before switching either to state 1 or 2, q is the detaching probability, and ζ is the ratio of the displacements in state 1 and 2. We use the variables q and ζ instead of k_{21} and k_{23} . We have $l_{23} = l_{12} q \zeta$ and $l_{21} = l_{12} (1 - q) \zeta$. Because diffusion in state 1 is slow compared to state 2, we have $\zeta \ll 1$. We further consider that the probability to switch from state 2 to 1 is much larger than the dissociation probability, such that $q \ll 1$. For $\zeta \ll 1$ and $q \ll 1$ we obtain from equation (4) and equation (6) the asymptotic

$$\bar{\tau} \approx \sqrt{2} \frac{L}{\sigma_1} \left(1 + \sqrt{\frac{\zeta}{q}} \right) \left(\frac{1}{k_{12}} + \frac{\sigma_1^2}{2D_2 \zeta} + \frac{q}{k_{32}} \right). \quad (8)$$

For fixed σ_1 , D_2 , k_{12} and k_{32} , the minimum of $\bar{\tau}$ with respect to (q, ζ) is

$$\bar{\tau} = \frac{\sqrt{2}L}{\sigma_1 k_{12}} (1 + \gamma \zeta^2) \quad (9)$$

achieved at $\gamma \zeta = \sqrt{\frac{2}{\delta}}$ and $\gamma^2 q \zeta = 1$, where $\gamma = \sqrt{\frac{2D_2}{k_{32} \sigma_1^2}}$ and $\delta = \frac{k_{32}}{k_{12}} \gamma = \frac{k_{32}}{k_{12}} \sqrt{\frac{2D_2}{k_{32} \sigma_1^2}}$. Interestingly, whereas the optimal rate k_{21} depends on the properties of state 1, we find for k_{23} the optimal value $k_{23} = \frac{D_2}{D_1} q \zeta = \frac{D_2}{D_1} \frac{1}{\gamma^2} = k_{32}$, independent of the properties of state 1.

Search with two states only

To derive the MFPT with switchings between two states we set $k_{23} \rightarrow 0$. With $\kappa_1 = \infty$ and $\kappa_2 = 0$ (state 2 now corresponds to the bulk state without binding) we find

$$\bar{\tau} = \hat{L} l_{12} \left(\frac{l_{21}}{\mu_2} \frac{\hat{L}}{3} + \frac{l_{12}}{\mu_2} \frac{1}{\sqrt{\mu_2}} \right) \left(\frac{1}{k_{12}} + \frac{1}{k_{21}} \right) \quad (10)$$

with $\mu_2 = l_{12} + l_{21}$. For $k_{12} \rightarrow 0$ we recover $\bar{\tau} = \frac{L^2}{3D_1}$. With uniform redistributions in state 2 we get ($l_{21} = 0$)

$$\bar{\tau} = \sqrt{2} \frac{L}{\sigma_1} \left(\frac{1}{k_{12}} + \frac{1}{k_{21}} \right). \quad (11)$$

equation (11) as a function of k_{12} has a minimum for $k_{12} = k_{21}$ (compare with $k_{23} = k_{32}$ obtained with 3 states).

Results

We present results that we compare to experimental measurements for a dimeric Lac repressor search in *E. coli* bacteria. We use a DNA length $2L = 4.8 \times 10^6$ bp [13], a TF attaches to the DNA from the bulk after an average time $k_{32}^{-1} = 1.4$ ms [13, 38], and the diffusion constant in state 2 is $D_2 = 2 \mu\text{m}^2 \text{s}^{-1}$ ($D_2 = 1.7 \times 10^7 \text{ bp}^2 \text{s}^{-1}$) [11, 13]. We keep these values fixed throughout the following analysis and we focus on investigating the impact of the remaining parameters.

Search scenario with conformational changes

A TF is freely diffusing in the bulk (state 3) and attaches to the DNA with a Poissonian rate k_{32} (figure 1). We consider that the association position follows a Gaussian distribution with variance σ_3^2 centered around the previous dissociation point. σ_3 is the correlation distance between subsequent detaching and attaching positions. Hence, σ_3 is an effective parameters that implicitly depends on the DNA configuration and on coiling. For example, a uniform re-attaching distribution obtained for $\sigma_3 = \infty$ is usually attributed to a highly packed DNA conformation [18, 32, 35, 39–41]. By varying σ_3 we can investigate how the DNA conformation affects the MFPT. We assume that a TF switches between a stable and an unstable protein conformation. The lifetime of the unstable conformation ξ^{-1} is short such that a TF quickly returns to its stable conformation after a spontaneous conformation change. When a TF is attached to the DNA and in the stable conformation (state 2) it non-specifically interacts with the DNA backbone and diffuses in a smooth potential well with non-specific energy E_{ns} and fast diffusion constant D_2 . In state 2 a TF can either dissociate from the DNA with rate k_{23} , or switch to the unstable conformation (state 1) with rate k_{21} . The unstable conformation allows for additional specific TF-DNA interactions that modify the residence time k_{12}^{-1} in state 1. We use the Arrhenius like relation $k_{12} = \xi e^{-\Delta E}$, where $\Delta E = E_{\text{ns}} - E$ (in units of $k_B T$). We use a maximal target affinity in state 1, $\chi_1 = \infty$, in which case the search is finished when a TF reaches the target in state 1 for the first time. In state 2, the outcome at the target depends on the affinity χ_2 (respectively the dimensionless parameter κ_2). The search is over for $\kappa_2 = \infty$, in which case a TF has maximal target affinity already in state 2. In the opposite case $\kappa_2 = 0$ a TF has no indication in state 2 that it has reached the target site and there is a probability that he misses the target and detaches

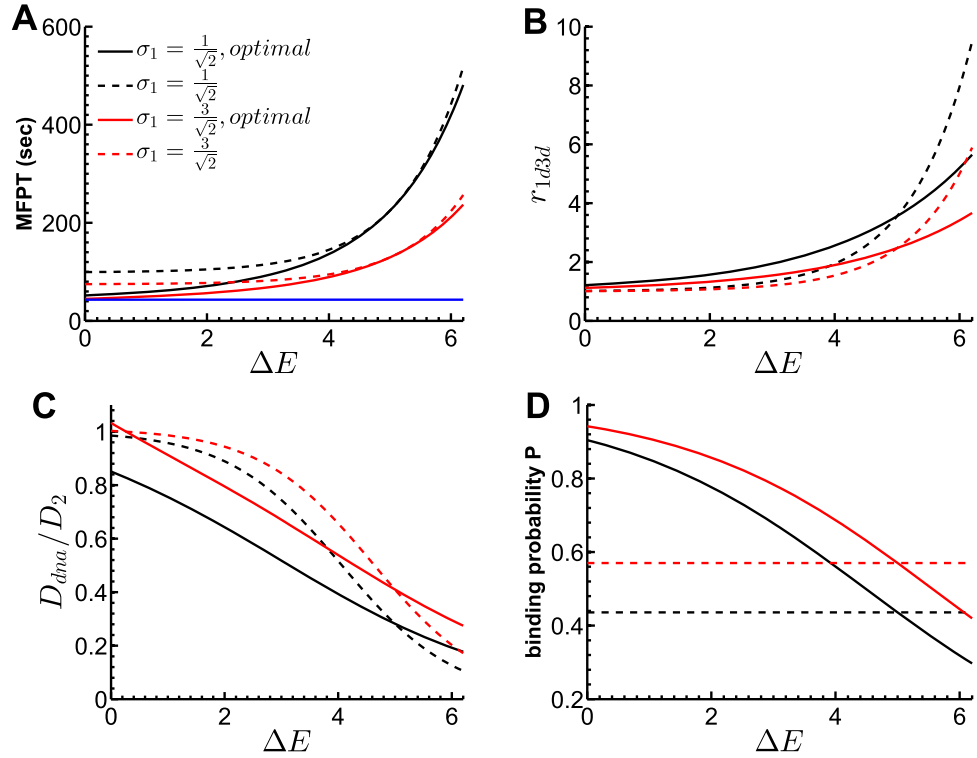


Figure 2. Search with no target affinity in state 2 ($\kappa_2 = 0$) and uniform redistributions ($\sigma_3 = \infty$). (A) MFPT for optimal and non-optimal searches as a function of the specific binding strength for two values of σ_1 ($k_{12} = \xi e^{-\Delta E}$, $\xi = 10^7 \text{ s}^{-1}$). For $\sigma_1 = 1/\sqrt{2}$ a TF is immobile in state 1 and scans only a single bp, for $\sigma_1 = 3/\sqrt{2}$ it scans three bp. The optimal MFPT is computed with equation (6), the non-optimal with equation (5). The blue line corresponds to the MFPT for a two-states switching process between state 2 and 3 with an absorbing target in state 2 ($\kappa_2 = \infty$). Energies are in units of $k_B T$. The rest of the parameters are: $\kappa_1 = \infty$, $k_{32}^{-1} = 1.4 \text{ ms}$, $D_2 = 2 \mu\text{m}^2 \text{ s}^{-1}$, $k_{23} = k_{32}$. The value of k_{21} for the non-optimal search equals the optimal value computed with $\Delta E = 5$. (B) Ratio of the time spent associated with the DNA compared to freely diffusing in the bulk. (C) Apparent diffusion constant for sliding along the DNA. (D) Probability P to bind to the target before dissociation when arriving at the target site.

without binding. Because switching to state 1 at the target site ends the search, by varying κ_2 we can explore the impact of induced switchings at the target site.

We use constant switching rates k_{21} , k_{23} and k_{32} . This is valid for a spatially homogenous DNA and a homogenous non-specific interaction in state 2. In contrast, k_{12} and σ_1 depend on the specific binding energy E and are therefore not constant along the DNA. To account for this, we first derive results using a constant E corresponding to a homogenous DNA, and in a subsequent step we average using a Gaussian distribution for E . Because of strong specific interactions, we focus on displacements σ_1 that are small. The lower bound for σ_1 is reached when a TF becomes immobilized in state 1. However, this does not correspond to $\sigma_1 = 0$, because a TF at least scans the bp it binds to. A non-zero σ_1 also accounts for stochastic fluctuations in the DNA position due to the switching process. By noting that the MSD of the maximum displacement of a diffusion process is $2\sigma_1^2$, we use $\sigma_1 = \frac{1}{\sqrt{2}}$ to model the limiting case where a TF becomes immobilized in state 1 and scans only a single bp.

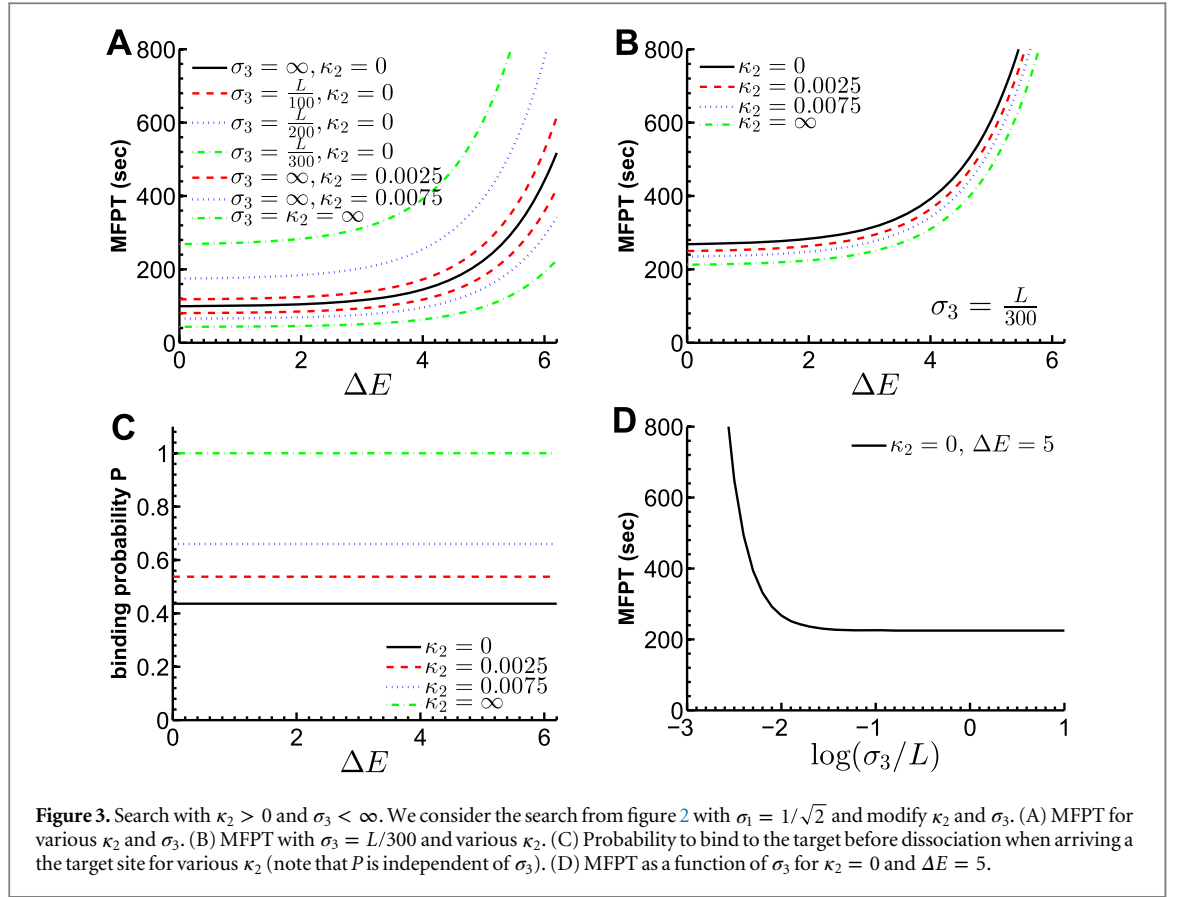
To facilitate the comparison with experimental data, we introduce the following parameters that characterize various properties of a search process:

$$\begin{aligned} \tau_{\text{dna}} &= \frac{1}{k_{23}} + \frac{k_{21}}{k_{23}} \frac{1}{k_{12}}, & r_{1d3d} &= k_{32} \tau_{\text{dna}}, \\ \sigma_{\text{dna}}^2 &= \sigma_2^2 \frac{1}{q} + \sigma_1^2 \frac{1-q}{q}, & D_{\text{dna}} &= \frac{\sigma_{\text{dna}}^2}{2\tau_{\text{dna}}}. \end{aligned} \quad (12)$$

τ_{dna} is the average time a TF stays bound to the DNA before detaching; r_{1d3d} is the ratio of the time bound to the DNA to diffusing in the cytoplasm; σ_{dna}^2 is the mean square displacement along the DNA before detaching; D_{dna} is the effective diffusion constant for sliding.

Search with uniform redistributions and no target affinity in state 2

We start by analyzing search processes as a function of the switching rate k_{12} with no target affinity in state 2 ($\kappa_2 = 0$) and uniform redistributions in state 3 (figure 2). We write k_{12} as function of the binding strength, $k_{12} = \xi e^{-\Delta E}$, and plot quantities as a function of ΔE . We use the basal rate $\xi = 10^7 \text{ s}^{-1}$, which is similar to the attempt frequency 10^8 s^{-1} used in [18], or 10^6 s^{-1} from [39]. At this stage the exact value of ξ is not important to show the behaviour as a function of ΔE . For example, a smaller value for ξ would shift the origin of the ΔE -axis to the right in figures 2 and 3, but otherwise does not affect the graphs. Later on we will



estimate a more appropriate value for ξ by considering a Gaussian binding energy distribution.

We compare optimal and non-optimal searches for $\sigma_1 = \frac{1}{\sqrt{2}}$ (1 bp is scanned in state 1) and $\sigma_1 = \frac{3}{\sqrt{2}}$ (up to 3 bp are scanned in state 1). The optimal search is characterized by $k_{23} = k_{32}$ and a rate k_{21} that is a function of ΔE and σ_1 (see equation 9). For non-optimal searches we use $k_{23} = k_{32}$ and a rate k_{21} that is independent of ΔE , since the properties of state 1 should not affect the switching rate k_{21} in state 2. We use the optimal value for k_{21} computed with $\Delta E = 5$ (hence, $k_{21} = 1.72 \times 10^5 \text{ s}^{-1}$ for $\sigma_1 = \frac{1}{\sqrt{2}}$ and $k_{21} = 9.92 \times 10^4 \text{ s}^{-1}$ for $\sigma_1 = \frac{3}{\sqrt{2}}$), which gives a fast search also with large ΔE . We use two different values for $\sigma_1 = \frac{1}{\sqrt{2}}$ and $\sigma_1 = \frac{3}{\sqrt{2}}$ to facilitate the comparison between optimal and non-optimal curves: in this case the optimal and non-optimal MFPT coincide for $\Delta E = 5$ (figure 2(a)).

Figure 2(a) shows that even an immobilized TF in state 1 can have a MFPT that is compatible with the experimental finding $\sim 350 \text{ s}$ [11, 13]. The MFPT is faster for $\sigma_1 = \frac{3}{\sqrt{2}}$ because more DNA is scanned during the same residence time in state 1. Interestingly, the MFPT varies only very little as a function of ΔE up to values $\Delta E \sim 5$ (figure 2(a)), suggesting that the search is insensitive to a large part of binding energy fluctuations. The asymptotic value of the optimal MFPT for small ΔE corresponds to a two states process where a TF switches between state 2 and 3 and has

maximal target affinity in state 2 (figure 2(a), blue curve). This is consistent with results from [37] showing that a switching process can have a fast MFPT even if the searcher can only bind in the slow state.

For an optimal search process with only two states (bulk and one sliding state), a TF spends an equal amount of time in the bulk and associated to the DNA. This is not any more the case for an optimal three states process (figure 2(b)). Although we have $k_{23} = k_{32}$, which is similar to the condition for a two states process, because of switchings to state 1, a TF spends much more time bound to the DNA. For an optimal search we have $r_{1d3d} = 1 + \sqrt{2}\delta$, where δ is defined after equation 9. For $\Delta E = 6$ and $\sigma_1 = \frac{1}{\sqrt{2}}$ we obtain $r_{1d3d} \approx 10$, which is similar to *in vivo* findings that a dimeric Lac repressor spends 90% of the search time bound to the DNA [13]. The effective diffusion constant for sliding D_{dna} decreases as specific binding becomes stronger (figure 2(c)). For an optimal search we compute $D_{\text{dna}} \approx D_2 / r_{1d3d}$. Thus, for $r_{1d3d} = 10$ we obtain $D_{\text{dna}} \approx 0.2 \frac{\mu\text{m}^2}{\text{s}}$, which is around 4 times larger than values estimated from single molecule tracking experiments on flow stretched DNA [13]. However, such a value is in good agreement with results from molecular dynamics simulations for a Lac dimer [42], and with an apparent 1D diffusion constant $D_{\text{eff}} \sim 0.4 \frac{\mu\text{m}^2}{\text{s}}$ estimated in [13]. Moreover, a large range of variability is observed for the 1D diffusion constant of a Lac repressor on elongated DNA

estimated from single molecule imaging techniques [14]. Whereas D_{dna} strongly depends on k_{12} , the sliding distance σ_{dna} is not affected by the residence time in state 1 (if σ_1 remains unchanged) and is determined by diffusion in state 2 and the detaching rate k_{23} . For an optimal search with $k_{23} = k_{32}$, by neglecting σ_1 , we obtain $\sigma_{\text{dna}} \approx \sqrt{\frac{2D_2}{k_{23}}} \approx 220$ bp. This value is much larger than *in vivo* measurements for a Lac dimer around 40 bps [11, 25], but compatible with a value around 240 bps obtained from molecular dynamics simulations [42]. Similar to D_{dna} , also for σ_{dna} a large experimental variability is observed using single molecule imaging techniques [14].

Finally, when arriving at the target site in state 2, a TF can as well detach without binding to the target [11, 25]. To characterize such events, we compute the conditional probability P to bind before detaching when arriving at the target site in state 2 (see SI equation 61). The probability depends on the affinity κ_2 , for example, for $\kappa_2 = \infty$ we have $P = 1$. For $\kappa_2 = 0$ the probability is not zero and it depends on the local switching dynamics. In general, P can be expressed as a function of the sliding distances independent of the switching rates. Thus, for constant σ_1 , P is independent of k_{12} or ΔE (figure 2(d)). For the optimal search process P depends on ΔE because k_{21} and therefore σ_2 vary with ΔE .

Search with finite redistributions and induced switchings at the target site

We proceed and study the effect of the DNA configuration and induced switchings at the target site by varying σ_3 and κ_2 . We consider a non-optimal search with $\sigma_1 = \frac{1}{\sqrt{2}}$. Lowering the correlation distance σ_3 up to $\sigma_3 \sim \frac{L}{100}$ (around 1% of the genome is correlated) has only little impact on the MFPT (figures 3(a) and (d)), but a further decrease strongly increases the MFPT (figure 3(d)). In contrast, induced switchings at the target site ($\kappa_2 > 0$) only moderately reduce the MFPT (figure 3(a) and (b)). For example, the difference in the MFPT between $\kappa_2 = 0$ and $\kappa_2 = \infty$ is much smaller compared to $\sigma_3 \sim \frac{L}{100}$ and $\sigma_3 \sim \frac{L}{300}$ (figure 3(b)). We conclude that a reduced coiling cannot be compensated by induced switchings at the target site. Although a larger κ_2 increases the probability to bind to the target (figure 3(c)), this has only a minor impact because P has already a value around 50% for $\kappa_2 = 0$.

Search with a Gaussian binding energy distribution

So far we used a constant rate k_{12} corresponding to a constant binding energy E . In reality, k_{12} depends on the DNA sequence and therefore on the DNA position x . To account for this, we consider a search with a Gaussian binding energy distribution $\rho(E)$ [18, 19]. We further consider the case where a TF is immobile in state 1 such that $\sigma_1 = 1/\sqrt{2}$ and the number of

switchings N_{12} (see equation 6) are both independent of x . Let $w(x)$ be the weight function that measures how often position x is visited during a search process compared to the average. For a uniform initial distribution, from symmetry considerations, we can deduce that $w(x) = 1$. In this case the MFPT is

$$\begin{aligned} \bar{\tau} &= N_{12} \left(\int \frac{w(x)}{k_{12}(x)} dx + \frac{1}{k_{21}} + \frac{k_{23}}{k_{21} k_{32}} \right), \\ &= N_{12} \left(\int \frac{\rho(E)}{k_{12}(E)} dE + \frac{1}{k_{21}} + \frac{k_{23}}{k_{21} k_{32}} \right). \end{aligned} \quad (13)$$

Hence, we can compute the MFPT with the average switching rate

$$\bar{k}_{12}^{-1} = \int \frac{\rho(E)}{k_{12}(E)} dE = \int \frac{\rho(E)}{\xi e^{E-E_{\text{ns}}}} dE. \quad (14)$$

With $E_{\text{ns}} \approx -11$ and a Gaussian distribution $\rho(E)$ with variance $\sigma = 5$ [18, 19] we obtain $\bar{k}_{12} \approx \xi e^{-\zeta}$ with $\zeta = \frac{\sigma^2}{2} + E_{\text{ns}} \approx 1.5$. Next we checked how the results in figure 2 with $\Delta E = \zeta = 1.5$ comply with the experimental data. We find that a much better level of agreement is achieved for $\Delta E \approx 5.5$. We note that the range of the ΔE axis in figure 2 depends on the value of ξ . Had we used a different value $\xi' = \xi e^{-5.5-\zeta} = \xi e^{-4} \approx 10^5 \text{ s}^{-1}$, the origin of the ΔE axis would be shifted to the right and the results for $\Delta E = 5.5$ in figure 2 would appear at $\Delta E = \zeta$. Thus, by using $\xi \sim 10^5 \text{ s}^{-1}$ instead of $\xi \sim 10^7 \text{ s}^{-1}$ we obtain a search scenario that is compatible with experimental data even in presence of a Gaussian energy distribution with large variance. Moreover, for $\xi \sim 10^5 \text{ s}^{-1}$ we also have $\xi \sim k_{21}$ and we have the Arrhenius like relation $\frac{k_{12}}{k_{21}} \approx e^{-(E-E_{\text{ns}})}$.

Discussion

We investigated a framework for facilitated diffusion with switchings between three states: a bulk state (state 3) and two states with sliding along the DNA (state 1 and 2) motivated by two TF protein conformations. The TF-DNA interaction and the target affinity depend on the conformation. From the bulk, a TF associates to the DNA with a Poissonian rate and a Gaussian distribution centered around his previous dissociation point. We analytically computed the MFPT, and the conditional probability to bind to the target before detaching when arriving at the target site. We further defined and computed various other properties that characterize the search process, e.g. sliding length, effective 1D diffusion constant or ratio of the time spent in 1D compared to 3D. We compared our results with experimental data for the dimeric Lac repressor search in *E. coli* bacteria. We investigated various properties of a search process that we now discuss in more detail.

Impact of the DNA conformation

It is still largely unclear how strongly the DNA conformation affects the search time [43–46]. In the literature one can find analytic results for two opposite cases: a rod-like DNA or a maximally coiled DNA where the re-attaching distribution is uniform. However, a systematic and consistent analysis where the impact of coiling is gradually changed is still outstanding. In our model, the association rate k_{32} and the correlation distance σ_3 are two effective parameters that implicitly depend on the DNA conformation. For fixed σ_3 , a higher attaching k_{32} decreases the MFPT, but only up to a lower limit that is attained for instantaneous jumps $k_{32} = \infty$ (equation 4). The MFPT is minimal for a uniform redistribution ($\sigma_3 \rightarrow \infty$, figure 3(a)), a scenario that is frequently used to analyze a facilitated diffusion process with a highly packed DNA conformation [18, 32, 35, 39–41]. We find that around 1% of the DNA has to become correlated by the 3D excursions in order to maintain such a fast MFPT (figure 3(a)). At lower correlation distances the search time is greatly prolonged (figure 3(d)). The value of σ_3 also determines how the search time scales as a function of the DNA length L . To show this we consider the number of switchings N_{12} that are necessary to find the target. N_{12} increases proportional to L for $\sigma_3 \rightarrow \infty$ (equation 5), and such a linear dependency is usually assumed in the literature. However, for finite σ_3 , the leading order asymptotic for large L is $N_{12} \sim L^2$ and not $N_{12} \sim L$. For finite L , a careful analysis is needed to determine whether the contribution $\sim L$ or $\sim L^2$ is dominant. For our analysis we considered that σ_3 and k_{32} are independent parameters, however, in general their values will be correlated. For example, let's consider stretched DNA. In this case, by assuming a correlation distance $\sigma_3 \sim L$, we find $N_{12} \sim L$. However, because the DNA is stretched and a TF is diffusing with diffusion constant D_3 in the bulk, we must at least have $k_{32}^{-1} \sim \sigma_3^2/D_3$. Finally, this leads to a MFPT that scales $\sim L^2$ and not $\sim L$. On the other hand, with strong coiling one might have $\sigma_3 \sim L$ with a fast rate k_{32} that is almost independent of σ_3 , such that the MFPT scales $\sim L$. We conclude that without coiling it is not possible to have a MFPT that scales $\sim L$. Coiling is permissive to obtain at the same time a large correlation distance σ_3 and a fast attaching rate k_{32} .

Impact of induced switchings

If switchings from state 2 to state 1 are induced at the target site, the spontaneous switching k_{21} can be reduced leading to a faster search because a TF spends more time in the fast state 2 [18, 26]. However, it is unclear which physical mechanism would provide such a specificity. We investigated the impact of induced switchings by varying the target affinity in state 2 (κ_2). We find that a MFPT compatible with experimental data can be achieved without induced

switchings (figures 22(A) and 23(A)). We estimated that in this case a switching rate around $k_{21} \approx 10^5 \text{ s}^{-1}$ is necessary. This implies conformational changes in the submillisecond range, that have also been suggested in [12]. The rate k_{21} could be further reduced by assuming a larger sliding distance σ_1 . If spontaneous switchings to state 1 are fast, the conditional probability P to bind to the target before detaching is already large for $\kappa_2 = 0$ (figure 23(C)). In such a case additional induced switchings ($\kappa_2 > 0$) do not much affect the search time (figure 3(b)). Clearly, the impact of induced switchings would be much larger if P would be small for $\kappa_2 = 0$. For example, this could be achieved by lowering the target affinity in state 1 ($\kappa_1 < \infty$), or by reducing the switching rate k_{21} . With such conditions the MFPT would be strongly increased by blocking induced switchings. In general, for large $\kappa_2 = \infty$ the fastest MFPT is achieved by simply not switching to the slow state 1 ($k_{21} = 0$). But in this case we return to a two states model with a bulk and a fast sliding state, incompatible with a large binding energy variability.

Search in presence of a Gaussian binding energy profile

State 1 is characterized by the displacement σ_1 and the residence time k_{12}^{-1} . We analyzed the limiting case where a TF becomes immobilized in state 1 such that it scans only the bp to which it binds to ($\sigma_1 = 1/\sqrt{2}$). The rate $k_{12} = \xi e^{E-E_{\text{ns}}}$ depends on the specific energy E in state 1 and the non-specific energy E_{ns} in state 2. With $\xi = 10^5 \text{ s}^{-1}$, $E_{\text{ns}} = -11k_B T$ and a Gaussian distribution for E with variance $\sigma = 5k_B T$ we obtained a MFPT around 5–6 minutes, compatible with *in vivo* experimental data for the dimeric Lac repressor [13]. It is found that the Lac repressor dimer stays bound to the promoter for an average time τ_b around 5 min [47]. In our model this would correspond to a target energy $E = E_{\text{ns}} - \ln(\xi\tau_b) \approx -28k_B T$, compatible with data [18, 19]. At strong noncognate DNA sites with $E \sim -25k_B T$, a TF would be trapped only for a short time $k_{12}^{-1} = (\xi e^{-25+11})^{-1} \sim 12 \text{ s}$, which resolves the trapping problem [39]. The speed-stability paradox strongly relies on the assumption that a TF is found with high probability bound to the target at thermodynamic equilibrium. However, when the MFPT is of the order of minutes, such a high probability implies that a TF blocks the promoter for a very long time. This would impede a fast cellular response, and generate the opposite problem of how a promoter can get rid of a tightly bound TF.

Conclusion and prospects

In this work we presented a MFPT analysis for a facilitated diffusion search process with switchings between three states: a bulk state and two sliding states where the TF is attached to the DNA. The model is

microscopically motivated and describes the local dynamics using effective parameters. Parameter values have to be extracted from more detailed models of the TF-DNA interaction, or by fitting our analytic expressions to experimental data for the dimeric Lac repressor. We focused on a qualitative analysis of the model and we showed that the model predictions account for many features that are observed experimentally.

A major simplification of the current model is the fact that we reduce the impact of the 3D dynamics to a Poissonian association rate k_{32} and a Gaussian re-attaching distribution with width σ_3 . However, this simplification allowed to derive analytic results, which are important to precisely analyze the parameter space. Furthermore, we generalized results with uniform redistributions corresponding to $\sigma_3 = \infty$. Assuming that σ_3 is correlated to the amount of DNA coiling, we could systematically investigate the impact of the DNA conformation. However, polymer models show that the distribution of σ_3 is not a Gaussian but decays like a power law at large distances [28, 29, 48]. The re-entry distribution is also more complicated than a single exponential. It will be interesting to investigate in future work how more accurate assumptions for the 3D dynamics based on polymer models change the results presented here. Another interesting project is to compute the MFPT with Lévy flights in state 3 [41, 45].

Instead of using a single state for the 3D dynamics with complex distributions for attaching time and position, one could break down the 3D dynamics into several states with simpler distributions and enlarge the current model by additional states. Each state would account for different properties of the search process, e.g. hoppings, jumps, intersegment and inter-segmental transfers.

Author contributions

J R conceived and supervised the research; J C and J R performed the analysis; J R and J C wrote the paper.

Acknowledgments

J Cartailier acknowledges support from a PhD grant from the University Pierre et Marie Curie.

References

- [1] Zabet N and Adryan B 2012 Computational models for large-scale simulations of facilitated diffusion *Mol. Biosyst.* **8** 2815–27
- [2] Kolomeisky A 2011 Physics of protein–DNA interactions: mechanisms of facilitated target search *Phys. Chem. Chem. Phys.* **13** 2088–95
- [3] Tafvizi A, Mirny L and van Oijen A 2011 Dancing on DNA: kinetic aspects of search processes on DNA *ChemPhysChem* **12** 1481–9
- [4] Li G and Elf J 2009 Single molecule approaches to transcription factor kinetics in living cells *FEBS Lett.* **583** 3979–83
- [5] Halford S and Marko J 2004 How do site-specific DNA-binding proteins find their targets? *Nucleic Acids Res.* **32** 3040–52
- [6] von Hippel P H and Berg O G 1989 Facilitated target location in biological systems *J. Biol. Chem.* **264**(2) 675–8
- [7] Riggs A D, Bourgeois S and Cohn M 1970 The lac repressor–operator interaction: III. Kinetic studies *J. Mol. Biol.* **53** 401–17
- [8] Richter P and Eigen M 1974 Diffusion controlled reaction rates in spheroidal geometry application to repressor–operator association and membrane bound enzymes *Biophys. Chem.* **2** 255–63
- [9] Berg O and Blomberg C 1976 Association kinetics with coupled diffusional flows. special application to the lac repressor–operator system *Biophys. Chem.* **4** 367–81
- [10] Berg O, Winter R and von Hippel P 1981 Diffusion-driven mechanisms of protein translocation on nucleic-acids: 1. Models and theory *Biochemistry* **20** 6926–48
- [11] Hammar P, Leroy P, Mahmutovic A, Marklund E, Berg O and Elf J 2012 The lac repressor displays facilitated diffusion in living cells *Science* **336** 1595–8
- [12] Tafvizi A, Huang F, Fersht A, Mirny L and van Oijen A 2011 A single-molecule characterization of p53 search on DNA *Proc. Natl Acad. Sci. USA* **108** 563–8
- [13] Elf J, Li G and Xie X 2007 Probing transcription factor dynamics at the single-molecule level in a living cell *Science* **316** 1191–4
- [14] Wang Y, Austin R H and Cox E 2006 Single molecule measurements of repressor protein 1D diffusion on DNA *Phys. Rev. Lett.* **97** 048302
- [15] Winter R and von Hippel P 1981 Diffusion-driven mechanisms of protein translocation on nucleic acids: II. The *Escherichia coli* lac repressor–operator interaction: equilibrium measurements *Biochemistry* **20** 6948–60
- [16] Slutsky M, Kardar M and Mirny L 2004 Diffusion in correlated random potentials, with applications to DNA *Phys Rev E* **69** 061903
- [17] Zwanzig R 1988 Diffusion in a rough potential *Proc. Natl Acad. Sci. USA* **85** 2029–30
- [18] Slutsky M and Mirny L 2004 Kinetics of protein–DNA interaction: facilitated target location in sequence-dependent potential *Biophys. J.* **87** 4021–35
- [19] Gerland U, Moroz J and Hwa T 2002 Physical constraints and functional characteristics of transcription factor–DNA interaction *Proc. Natl Acad. Sci. USA* **99** 12015–20
- [20] Leith J, Tafvizi A, Huang F, Uspal W, Doyle P, Fersht A, Mirny L and van Oijen A 2012 Sequence-dependent sliding kinetics of p53 *Proc. Natl Acad. Sci. USA* **109** 16552–7
- [21] Kalodimos C, Biris N, Bonvin A, Levandoski M, Guennegues M, Boelens R and Kaptein R 2004 Structure and flexibility adaptation in nonspecific and specific protein–DNA complexes *Science* **305** 386–9
- [22] Horton J, Liebert K, Hattman S, Jeltsch A and Cheng X 2005 Transition from nonspecific to specific DNA interactions along the substrate-recognition pathway of dam methyltransferase *Cell* **121** 349–61
- [23] Viadiu H and Aggarwal A 2000 Structure of BamHI bound to nonspecific DNA: a model for DNA sliding *Mol. Cell.* **5** 889–95
- [24] Albright R, Mossing M and Matthews B 1998 Crystal structure of an engineered cro monomer bound nonspecifically to DNA: possible implications for nonspecific binding by the wild-type protein *Protein Sci.* **7** 1485–94
- [25] Mahmutovic A, Berg O and Elf J 2015 What matters for lac repressor search *in vivo*-sliding, hopping, intersegment transfer, crowding on DNA or recognition? *Nucleic Acids Res.* **43** 3454–64
- [26] Zhou H-X 2011 Rapid search for specific sites on DNA through conformational switch of nonspecifically bound proteins *Proc. Natl Acad. Sci. USA* **108** 8651–6
- [27] Murugan R 2010 Theory of site-specific DNA-protein interactions in the presence of conformational fluctuations of DNA binding domains *Biophys. J.* **99** 353–9

- [28] Díaz de la Rosa M, Koslover E, Mulligan P and Spakowitz A 2010 Dynamic strategies for target-site search by DNA-binding proteins *Biophys. J.* **98** 2943–53
- [29] Mirny L, Slutsky M, Wunderlich Z, Tafvizi A, Leith J and Kosmrlj A 2009 Spatial effects on the speed and reliability of protein–DNA search *J. Phys. A: Math. Theor.* **42** 434013
- [30] Hu L, Grosberg A and Bruinsma R 2008 Are DNA transcription factor proteins Maxwellian demons? *Biophys. J.* **95** 1151–6
- [31] Bauer M and Metzler R 2012 Generalized facilitated diffusion model for DNA-binding proteins with search and recognition states *Biophys. J.* **102** 2321–30
- [32] Reingruber J and Holcman D 2011 Transcription factor search for a DNA promoter in a three-states model *Phys. Rev. E* **84** 020901
- [33] Hu L, Grosberg A and Bruinsma R 2009 First passage time distribution for the 1D diffusion of particles with internal degrees of freedom *J. Phys. A: Math. Theor.* **42** 434011
- [34] Veksler A and Kolomeisky A 2013 Speed-selectivity paradox in the protein search for targets on DNA: is it real or not? *J. Phys. Chem. B* **117** 12695–701
- [35] Coppey M, Bénichou O, Voituriez R and Moreau M 2004 Kinetics of target site localization of a protein on DNA: a stochastic approach *Biophys. J.* **87** 1640–9
- [36] Reingruber J and Holcman D 2010 Narrow escape for a stochastically gated Brownian ligand *J. Phys.: Condens. Matter* **22** 065103
- [37] Reingruber J and Holcman D 2009 Gated narrow escape time for molecular signaling *Phys. Rev. Lett.* **103** 148102
- [38] Malherbe G and Holcman D 2010 The search for a DNA target in the nucleus *Phys. Lett. A* **374** 466–71
- [39] Bénichou O, Kafri Y, Sheinman M and Voituriez R 2009 Searching fast for a target on DNA without falling to traps *Phys. Rev. Lett.* **103** 138102
- [40] Sheinman M and Kafri Y 2009 The effects of intersegmental transfers on target location by proteins *Phys. Biol.* **6** 016003
- [41] Lomholt M, Ambjörnsson T and Metzler R 2005 Optimal target search on a fast-folding polymer chain with volume exchange *Phys. Rev. Lett.* **95** 260603
- [42] Marklund E, Mahmutovic A, Berg O, Hammar P, van der Spoel D, Fange D and Elf J 2013 Transcription-factor binding and sliding on DNA studied using micro- and macroscopic models *Proc. Natl Acad. Sci. USA* **110** 19796–801
- [43] Koslover E, Díaz de la Rosa M and Spakowitz A 2011 Theoretical and computational modeling of target-site search kinetics *in vitro* and *in vivo* *Biophys. J.* **101** 856–65
- [44] Lomholt M, van den Broek B, Kalisch S, Wuite G and Metzler R 2009 Facilitated diffusion with DNA coiling *Proc. Natl Acad. Sci. USA* **106** 8204–8
- [45] Lomholt M, Koren T, Metzler R and Klafter J 2008 Lévy strategies in intermittent search processes are advantageous *Proc. Natl Acad. Sci. USA* **105** 11055–9
- [46] Hu T, Grosberg A and Shklovskii B 2006 How proteins search for their specific sites on DNA: The role of DNA conformation *Biophys. J.* **90** 2731–44
- [47] Hammar P, Walldén M, Fange D, Persson F, Baltekin O, Ullman G, Leroy P and Elf J 2014 Direct measurement of transcription factor dissociation excludes a simple operator occupancy model for gene regulation *Nat. Genet.* **46** 405–8
- [48] Fudenberg G and Mirny L 2012 Higher-order chromatin structure: bridging physics and biology *Curr. Opin. Genet. Dev.* **22** 115–24