Sliding and jumping of single EcoRV restriction enzymes on non-cognate DNA

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ABSTRACT
The restriction endonuclease EcoRV can rapidly locate a short recognition site within long non-cognate DNA using ‘facilitated diffusion’. This process has long been attributed to a sliding mechanism, in which the enzyme first binds to the DNA via nonspecific interaction and then moves along the DNA by 1D diffusion. Recent studies, however, provided evidence that 3D translocations (hopping/jumping) also help EcoRV to locate its target site. Here we report the first direct observation of sliding and jumping of individual EcoRV molecules along nonspecific DNA. Using fluorescence microscopy, we could distinguish between a slow 1D diffusion of the enzyme and a fast translocation mechanism that was demonstrated to stem from 3D jumps. Salt effects on both sliding and jumping were investigated, and we developed numerical simulations to account for both the jump frequency and the jump length distribution. We deduced from our study the 1D diffusion coefficient of EcoRV, and we estimated the number of jumps occurring during an interaction event with nonspecific DNA. Our results substantiate that sliding alternates with hopping/jumping during the facilitated diffusion of EcoRV and, furthermore, set up a framework for the investigation of target site location by other DNA-binding proteins.

INTRODUCTION
Accelerated target location by site-specific DNA-binding proteins has motivated numerous experimental and theoretical studies for over thirty years (1–7). It is now widely accepted that, in order to reach their target site, proteins first translocate along nonspecific DNA, i.e. move along DNA that does not contain any specific site. The mechanism underlying this so-called ‘facilitated diffusion’, however, is still under debate. Sliding, a process that involves linear diffusion along nonspecific DNA, has long been considered as the main mechanism of facilitated diffusion (8). Apart from allowing proteins to carefully scan the DNA, one-dimensional diffusion, by reducing the dimensionality of the space to be explored, can speed up target site location compared with 3D search in solution (9,10). Supporting the linear diffusion hypothesis, recent single-molecule experiments, performed both in vitro (11–16) and in vivo (17), have shown that various proteins can slide along DNA. Alternatively, fast target location can be ascribed to hopping/jumping (2,18). Hopping and jumping stem from the same mechanism, which involves dissociation, 3D diffusion and re-association of the protein to the same DNA molecule. The distinction between the two processes is based on the location of the re-association site, which is either close to the dissociation location (hopping), or far from it (jumping) (2,18). It was realized early that re-associations of a protein after dissociation from the DNA are highly probable (2), and, recently, this hypothesis was supported by bulk experiments demonstrating a significant contribution of 3D

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

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translocations to target site location (18). Besides, jumping allows a protein to rapidly reach DNA sites which are far from the initial binding site, and thus can be considered as complementary to sliding, an inefficient mode of searching over long distances (19). Hopping/jumping may also be more appropriate for an in vivo search, as the large number of proteins bound to the DNA makes a sliding motion over large distances almost impossible (20). The most effective target search might actually consist of a balanced combination of both sliding and hopping/jumping, as suggested by recent theoretical studies (21–26). However, in contrast to sliding, 3D translocations have so far not been observed in single-molecule experiments.

Among site-specific DNA-binding proteins, type II restriction enzymes are well-suited for the investigation of facilitated diffusion by in vitro kinetic studies (27,28). Specifically, EcoRV has been the subject of intensive research. Translocation of the enzyme from nonspecific DNA to its recognition site was initially attributed to sliding (29). This hypothesis was corroborated by experiments demonstrating that target location is accelerated if the length of nonspecific DNA flanking the EcoRV recognition site is increased (30) and by the study of target search by EcoRV in vivo (31). Sliding is also supported by the crystal structure of EcoRV in complex with nonspecific DNA (32), in which the enzyme has an open conformation that sustains the possibility of a linear diffusion along the DNA (33). In addition, facilitated diffusion of EcoRV has been addressed in experiments involving DNA with two recognition sites in close proximity, the interpretation of which suggested that hopping/jumping plays a major role in the process (34). However, alternative interpretation of the experimental data has been proposed which emphasized the role of sliding (35). These conflicting conclusions probably stem from the use of different models that involve successive stages to describe EcoRV-DNA interactions (interaction with nonspecific DNA, association to the target, cleavage of the DNA and subsequent release from the substrate), the features of which are not known in all details. Besides, experimental evidence for hopping/jumping of EcoRV has been provided by recent experiments involving a DNA catenane substrate (36).

A single-molecule approach is an appealing strategy for tackling the question of how EcoRV finds its target site. Recently, the interaction of endonucleases with the DNA has been studied using manipulations of individual DNA molecules with tweezers (37–39). These experiments, however, relied on measurements of forces and DNA length changes, and therefore are not adapted to investigations regarding facilitated diffusion. In contrast, fluorescence microscopy makes it possible to track a labelled enzyme and to observe its translocation path on a DNA molecule. Moreover, the study of facilitated diffusion by single-molecule fluorescence microscopy is not biased by enzyme cleavage kinetics, whereas biochemical experiments usually require DNA cleavage.

We report in this article the direct observation of single fluorescently labelled EcoRV interacting with elongated DNA molecules by Total Internal Reflection Fluorescence Microscopy (TIRFM, see Figure 1A). In order to investigate solely the facilitated diffusion of the enzyme, we used DNA that does not contain any EcoRV cleavage site. Recording the protein trajectories allowed us to establish the sliding of EcoRV along DNA and to determine the linear diffusion coefficient of the enzyme. Concurrently, we observed large translocations of the proteins along the DNA which were orders of magnitude faster than expected for sliding. Experiments performed under flow strongly suggest that these large jumps are due to free 3D excursions. Furthermore, we performed numerical simulations based on a 3D-diffusion model which accounted for both the frequency and the length distribution of the large jumps. We also performed experiments at different salt concentrations, the results of which indicate that not only large jumps, but also smaller jumps that cannot be detected by optical means, may alternate with sliding phases during DNA–EcoRV interaction.

**Figure 1.** Single-molecule set-up for the study of the facilitated diffusion of EcoRV along nonspecific DNA. (A) Biotinylated DNA molecules are attached at both ends to a streptavidin-coated surface. The molecules are in an elongated conformation, but free to fluctuate. Proteins are visualized using Total Internal Reflection Fluorescence Microscopy. (B) EcoRV tertiary structure displaying the Cy3B-labelling performed at position 58, remote from the DNA-binding site. (C) The accumulation of the fluorescence signal of hundreds of enzymes is used to visualize the elongated DNA (movie of duration ≈150 s, pixel size 126 nm, exposure time 20 ms). Note that, for better visualization, the video sequence was recorded at much higher enzyme concentration than used during single-molecule experiments. Due to the passivation of the surface, only a few proteins stuck to the surface, even at high concentration of enzymes. (D) A single frame from the movie depicts four enzymes bound to the DNA. The bright spot in the lower left corner stems from enzyme interactions with a DNA bound by only one end to the surface.
MATERIALS AND METHODS

Enzyme preparation and labelling

Wild type EcoRV is a homodimeric protein that contains one cysteine residue per subunit located close to the core of the protein. We prepared an EcoRV C21S/K58C variant in which the native cysteine residue (C21) was substituted by a serine residue and a single cysteine was inserted at position 58 (33), far from the active center (Figure 1B). Labelling was achieved using a Cy3B-maleimide Mono-Reactivate pack (Amersham Bioscience). Unreacted Cy3B-maleimide was removed using a Zeba desalt Spin column (Pierce). The labelling efficiency was about 0.4, estimated using $\varepsilon_{559}^{\text{Cy3B}} = 1.3 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$, $\varepsilon_{280}^{\text{Cy3B}} = 1.5 \times 10^4 \text{M}^{-1} \text{cm}^{-1}$ and $\varepsilon_{280}^{\text{EcoRV}} = 4.84 \times 10^4 \text{M}^{-1} \text{cm}^{-1}$. The EcoRV variant as well as the fluorescent-labelled protein displayed negligible loss of cleavage activity compared to the wildtype enzyme (data not shown). Observation of labelled enzymes stuck to the surface revealed that photobleaching occurred in a maximal number of two steps, which is consistent with the presence of one or two dyes on the protein complex. Single-molecule measurements were performed at pH 7.5 in 20 mM buffer, 10 mM MgCl$_2$, 10–60 mM NaCl, 1 mM DTT and 0.02% v/v blocking reagent (Roche Diagnostics). Experiments were carried out in four different buffers: HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid), PIPES (1,4-Piperazine-diethanesulfonic acid), PB (sodium phosphate) or Tris (Tris(hydroxymethyl)aminomethane acetate salt), all purchased from Sigma-Aldrich.

DNA preparation and stretching

T7 bacteriophage DNA (Biocentric), which does not contain any recognition site for EcoRV, was cleaved with BsmBI (New England Biolabs). The longest fragment (~8.2 kbp) was ligated to two 500 bp PCR fragments, each containing about 70 biotin-modified uracil bases (Roche Diagnostics), according to a previously published method (40). Glass coverslips were silanized with 0.1% v/v aminopropyl-triethoxysilane (Sigma Chemical) in acidic ethanol. A flow cell was made by adhering, via a parafilm spacer, a silane-coated coverslip to a microscope slide into which two holes had been drilled as inlet and outlet. Biotinylated Blocking Reagent (1 mg/ml, prepared by reaction of 2 μl of Sulfo-NHS-LC- LC-Biotin (Pierce) at 100 mg/ml in DMSO with 200 μl of 2 mg/ml Blocking Reagent (Roche Diagnostics) in PIPES 20 mM, NaCl 100 m, pH 6.8), then streptavidin (0.1 mg/ml in PIPES 20 mM, pH 6.8), were incubated in the cell for 10 min. Subsequent incubation of the biotinylated DNA for a few seconds resulted in the attachment of DNA molecules to the surface by one end. Application of a fast flow (~70 μm/s near the surface) induced the elongation of the DNA and the binding of the second biotinylated DNA end to the surface. The elongated molecules were then stained with a DNA groove-binding dye (SybrGold, Invitrogen) and observed by fluorescence microscopy. We found that the DNA molecules were elongated to about 70% of the contour length. Observation of transverse DNA fluctuations ensured that surface attachment occurred solely via the biotinylated ends (41), while the main part of the DNA was freely accessible in solution. Elongated DNA molecules were stable for hours, and we never observed a detachment of the ends from the surface. In order to reduce nonspecific interactions between the proteins and the surface, residual streptavidin was passivated with biotinylated Blocking Reagent (0.1 mg/ml in 20 mM PIPES, pH 6.8, 50 mM NaCl) for 10 min.

Optical set-up

The flow cell was placed on an inverted microscope (Olympus IX70) equipped with a 60X oil-immersion objective (NA = 1.45, Olympus). After staining, the elongated DNA molecule were located with the aid of a mercury lamp using appropriate excitation and emission filters (480DF40 and longpass 505 LP, respectively, Omega Optical). After recording the positions of the elongated DNA molecules, the dye was removed for further measurements by flushing the flow cell with 2 ml of buffer containing 50 mM MgCl$_2$. We used TIRFM to detect the labelled enzymes, using for excitation a laser at 532 nm with a light intensity of 100 W/cm$^2$. Fluorescent light was collected via a dichroic mirror (560DRLP, Omega Optical) in combination with a longpass filter (565ALP, Omega Optical) and imaged on an EMCCD Camera (Ixon, Andor Technology) with a 20 ms exposure time. We used a home-made program written in MatLab to fit the point-spread function of the fluorescent spots with a two-dimensional Gaussian function, and to derive the Mean Square Displacement (MSD) from the enzyme trajectories.

RESULTS

Single-molecule assay

We attached biotinylated DNA molecules in an elongated conformation to a streptavidin-coated surface (Figure 1A) using a technique similar to those described in previous publications (41,42). We used a modified T7 DNA fragment (9.2 kbp) which was elongated to 70% of its contour length. EcoRV was labelled with Cy3B at a specific cysteine residue remote from the active center of the protein (Figure 1B). After injection of the fluorescently labeled enzymes in the flow cell, the flow was stopped. At a concentration of EcoRV in the nM range, we detected several enzymes simultaneously interacting with the DNA template, while rarely nonspecific interactions with the surface were observed (Figure 1C and D, and Supplementary Data 3). However, for single-molecule analysis, we reduced the enzyme concentration to 5–20 pM in order to observe, on average, less than one enzyme on the DNA at a given time (Supplementary Data 4 and 5). The position of the enzyme could be determined in each frame, thereby allowing us to reconstruct the enzyme trajectory. The localization accuracy, which was limited by the number of photons accumulated during the exposure time (20 ms), was about 30 nm (Supplementary Data). We checked that the fluorescence signal was due to a single enzyme and that the dye used for labelling did not influence the enzyme dynamics (Supplementary Data).
At low NaCl concentration (10 mM), single enzymes interacted with the DNA on a time-scale of seconds, yielding long enzyme trajectories (typically more than 50 frames). An interaction event ended when we could not detect the enzyme for more than two consecutive frames, either because the enzyme dissociated from the DNA or because of the photobleaching of the dye. We considered for further analysis only interaction events longer than 30 frames (600 ms). The mean time between two consecutive interaction events was about 5–10 s. Since ensemble measurements had indicated an effect of the buffer on the cleavage kinetics (43), experiments were performed at pH 7.5 with four different buffers: HEPES, PIPES, sodium phosphate (PB) and Tris. For each buffer we recorded hundreds of DNA–EcoRV interaction events.

### Sliding of EcoRV

A typical single enzyme trajectory is shown in Figure 2A, which displays both the longitudinal (i.e. along the DNA) and transverse (i.e. perpendicular to the DNA and within the focal plane) positions of the enzyme as a function of time. Two processes contribute to the variations of the longitudinal position of the enzymes: the motion along the DNA and the thermal fluctuations of the elongated DNA template (Figure 2A). For each interaction event, we computed the MSD versus time of the enzyme to discriminate between sliding and DNA fluctuations (see Supplementary Data for the calculation of the MSD). The longitudinal and the transverse MSD calculated from a single enzyme trajectory are displayed in Figure 2B. While the transverse MSD is constant, the longitudinal MSD depends linearly on time, as expected for an enzyme sliding along DNA (12–15). The thermal fluctuations of the DNA only contribute as additional offsets to the MSD curves, since the exposure time (20 ms) is much larger than the correlation time of the transverse and longitudinal fluctuations, in the ms range (Supplementary Data). We used the time dependence of the longitudinal MSD and the value of the transverse MSD to determine the diffusion coefficients.

![Figure 2](http://nar.oxfordjournals.org/)
MSD (generally larger than $2 \times 10^{-3}$ nm$^2$) to discriminate enzymes interacting with the DNA from the enzymes sticking occasionally to the surface near the DNA, since in the last case both the longitudinal and transverse MSD are constant and below $10^{-2}$ nm$^2$ (Supplementary Data). For each interaction event, the diffusion constant $D_1$ was derived from the slope of the linearly fitted longitudinal MSD curve after correction for the DNA stretch rate (see Supplementary Data for the detail of the derivation of $D_1$). The histograms of $D_1$ deduced from single-molecule trajectories in PIPES and Tris are displayed in Figure 2C. For each buffer, we calculated the MSD averaged over all the analysed events (Figure 2D and Supplementary Data for the detail of the calculation). We obtained $D_1 = 1.1 \pm 0.2 \times 10^{-2}$ nm$^2$/s in PIPES (266 events) and $0.9 \pm 0.2 \times 10^{-2}$ nm$^2$/s in Tris (379 events). Similar diffusion constants were found in PB ($0.9 \pm 0.2 \times 10^{-2}$ nm$^2$/s, 740 events) and HEPES ($1.2 \pm 0.3 \times 10^{-2}$ nm$^2$/s, 162 events) (data not shown). These results show that the diffusion constant $D_1$ depends only weakly on the buffer.

**Jumps of EcoRV along DNA**

Some enzyme trajectories along the DNA exhibited large steps (up to 1.8 µm) within two consecutive frames (40 ms) (Figure 3A and B, and Supplementary Data 2, 6 and 7). Large steps were observed with equal probability in both directions. We focussed on steps larger than 200 nm, which are easy to detect since their lengths are larger than both the optical resolution of the microscope and the amplitude of longitudinal fluctuations in the enzyme trajectories before and after the step. The frequency of such large steps, i.e. the number of large steps per interaction event, ranged from 6% in HEPES to 16% in Tris. This frequency is difficult to explain considering a sliding motion with $D_1 \sim 10^{-2}$ nm$^2$/s. Indeed, the mean length $\langle l \rangle$ covered within $\Delta T = 40$ ms in a 1D Brownian motion is $\langle l \rangle = \sqrt{2D_1\Delta T} \approx 30$ nm, and a statistical analysis of the distribution of the lengths $l$ reveals that, regarding this value of $\langle l \rangle$, the probability of observing steps larger than 200 nm is extremely small (Supplementary Data). Therefore, these steps, which from now on will be called ‘large jumps’, were attributed to a translocation mechanism which differs from sliding. Large jumps cannot stem from a second enzyme associating immediately after dissociation of the first because, considering the association frequency of EcoRV with the DNA under our experimental conditions (5–10 per min), we estimated the probability of such an event to be smaller than 1% (Supplementary Data). Likewise, large jumps are unlikely to be due to transfers from the DNA to the surface or vice versa, since we could discriminate, via the MSD curves, enzymes interacting with the DNA from those stuck to the surface.

The lengths of the large jumps in different buffers are displayed in Figure 4A using normalized complementary cumulative distributions, which give the probability of observing jumps of length $s$ or larger as a function of $s$ during an interaction event. We chose to use complementary cumulative distributions because we focussed on large jumps and such distributions do not depend on the distribution of possible smaller jumps. In contrast to our results regarding the diffusion constant $D_1$, the occurrence of large jumps as well as the jump length distributions show a noticeable dependence on the buffer (Figure 4A). For PIPES, we found 26 jumps in 266 events (10%) compared to 59 jumps in 379 events for Tris (16%). The jump length distribution follows a similar trend with more than 50% of the large jumps spanning more than 500 nm in Tris, while only less than one third beyond this length are found in PIPES. The influence of the buffer on jump occurrence and distribution is further illustrated by the results obtained in HEPES (10 jumps in 162 events, i.e. 6%) and PB (109 in 740 events, i.e. 15%). Note that, in order to prevent alteration of the estimation of $D_1$ by the large jumps, the events that contained such a translocation were split into two trajectories (before and after the large jump), which were treated as independent events for the determination of $D_1$.

**Experiments under flow**

The observation of large jumps in our experiments denotes the existence of a fast translocation mechanism of the enzyme which differs from sliding. A probable mechanism involves dissociation of the enzyme from the DNA followed by re-association after a 3D diffusion. Since the
Changes in ionic conditions are known to modify the kinetics of DNA–protein interactions. In particular, an increase of the concentration of monovalent ions has been shown to strongly decrease the DNA–protein interaction time, while the rate of association to nonspecific DNA is only moderately affected (44). Salt changes are thus expected to affect the relative roles of sliding and hopping/jumping in facilitated diffusion (2,45) (Supplementary Data).

In order to address the effect of ionic conditions on EcoRV sliding and jumping, we performed experiments at NaCl concentrations ranging from 10 mM to 60 mM, the latter [NaCl] providing an ionic strength (~110 mM) comparable to physiological conditions. Salt-dependent experiments were carried out in PIPES. For each [NaCl], we recorded hundreds of interaction events, from which we derived the mean DNA–EcoRV interaction time.

3D diffusion coefficient $D_3$ for EcoRV is about $50 \mu m^2/s$ (see below), the distance covered by 3D motion within 40 ms can be in the $\mu m$ range, and thus 3D diffusion may account for the observed length of the large jumps. In principle, another mechanism could involve a fast 1D diffusion in which the enzyme stays bound to the DNA during a fast translocation. To discriminate between 1D and 3D mechanism, we modified the experimental set-up to apply a flow of enzyme solution perpendicular to the elongated DNA. The 3D movement of the enzyme and thus the jump lengths are likely to be affected by the drag of the flow, while it is not expected to influence a fast 1D diffusion during which EcoRV remains firmly bound to the DNA. Flow experiments were carried out in Tris, in which we previously observed the most frequent and largest jumps. When a flow of velocity $v = 70 \mu m/s$ was applied (see Supplementary Data for an estimation of the flow velocity), the number of jumps per event was reduced to about one half, i.e. jumps larger than 200 nm were observed in only 8% of the interaction events (39 jumps per 470 events) compared to 16% without flow (59 jumps in 379 events). In addition, the jump distribution was significantly shifted to smaller jump lengths, such that only 10% of the recorded jumps were longer than 700 nm compared to 30% in the experiments without flow (Figure 4B). The shift towards smaller jump lengths can be qualitatively accounted for by a simple 3D diffusion model that estimates the length of the jumps affected by the flow. For this purpose, we calculated the distance $l_D$ covered by 3D diffusion during a time $t$, $l_D \approx \sqrt{D_3/t}$, and the drift $l_v$ due to the flow during the same time, $l_v = vt$. The time after which the drift overcomes the diffusion (i.e., $l_v > l_D$) is approximately $D_3/v^2$. Thus, the distribution of the jump lengths is significantly affected for lengths larger than $D_3/v \approx 700 \mu m$, as observed experimentally. These results provide strong evidence that the large jumps are due to a 3D translocation of the enzymes.

**Effect of increasing salt concentration**

Changes in ionic conditions are known to modify the kinetics of DNA–protein interactions. In particular, an increase of the concentration of monovalent ions has been shown to strongly decrease the DNA–protein interaction time, while the rate of association to nonspecific DNA is only moderately affected (44). Salt changes are thus expected to affect the relative roles of sliding and hopping/jumping in facilitated diffusion (2,45) (Supplementary Data).

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The diffusion constants of the enzyme at different [NaCl] were derived from the averaged MSD (Figure 5A). At 20 mM NaCl, the diffusion constant was $D_1 = 1.2 \pm 0.1 \times 10^{-2} \mu m^2/s$ (217 events), i.e. similar to that obtained at 10 mM NaCl ($D_1 = 1.1 \pm 0.1 \times 10^{-2} \mu m^2/s$ (168 events)). For larger [NaCl], we observed a significant increase of the mean diffusion constant, $D_1 = 1.6 \pm 0.1 \times 10^{-2} \mu m^2/s$ at 40 mM NaCl (120 events) and $D_1 = 2.5 \pm 0.2 \times 10^{-2} \mu m^2/s$ at 60 mM NaCl (179 events) (Figure 5A and C). For each [NaCl], we also determined the complementary cumulative length distribution of the large jumps, i.e. larger than 200 nm. The number of large jumps during DNA–EcoRV interaction was found to decrease monotonously with increasing salt concentration (Figure 5B): at 60 mM NaCl, the number of large jumps was smaller by a factor 2.5 compared to 10 mM NaCl. The shape of the distribution, however, did not depend on the salt concentration (inset in Figure 5B).

**DISCUSSION**

### Linear diffusion coefficient

We derived from our experiments at low [NaCl] (i.e. in conditions where $D_1$ is not affected by small jumps, see below) a linear diffusion constant $D_1$ of approximately $10^{-2} \mu m^2/s$, similar to that reported in recent single-molecule experiments reporting the diffusion of other proteins along DNA [12–16]. $D_1$ is three orders of magnitude smaller than the 3D diffusion coefficient $D_{3}$, which is about $50 \mu m^2/s$ for EcoRV (see below). This drastic reduction is usually attributed to two factors: the hydrodynamics of the enzyme sliding along DNA and the modulation of the DNA–protein interaction potential during the 1D walk. If one models the enzyme by a sphere of radius $r$ that diffuses linearly along the DNA helix, the diffusion constant can be written as $D_1^{\text{hydro}} = 3h^2D_3/16\pi r^2$, where $h = 3.4 \text{ nm}$ is the pitch of the DNA helix [46]. Using Fluorescence Correlation Spectroscopy, we measured the hydrodynamic radius $r = 4.0 \pm 0.1 \text{ nm}$ of EcoRV-Cy3B in PIPES (Supplementary Data). This value yields $D_3^{\text{hydro}} = k_B T/6\pi \eta r = 54 \mu m^2/s$ at $T = 300 \text{ K}$ in water ($\eta = 10^{-3} \text{ Pa.s}$) and $D_1^{\text{hydro}} = 3.4 \mu m^2/s$. Additional reduction of the linear diffusion constant can be attributed to a modulation of the DNA–protein interaction during linear diffusion. Considering a sequence-dependent energy landscape of roughness $\sigma$, the diffusion constant $D_1$ reads [21]: $D_1 = D_1^{\text{hydro}} (1 + \beta \sigma^2/2)^{-1/2} \exp(-7\beta \sigma^2/4)$, with $\beta = 1/k_B T$. Using $D_1^{\text{hydro}} = 10^{-2} \mu m^2/s$, we found $\sigma = 1.6 k_B T$. This value is below $2k_B T$, which has been predicted to be the upper limit for accelerated target location involving 1D motion [21]. The relatively large value of $\sigma$ and the subsequent slow diffusion could be rationalized by the large contact region between EcoRV and the DNA, spanning at least 10 bp [32]. The diffusion constant we measured is somewhat below that derived from kinetic experiments with EcoRV, ranging from $3 \times 10^{-2} \mu m^2/s$ to $10^{-1} \mu m^2/s$ [30,47]. These studies, however, only considered sliding in the model used for fitting the kinetic data,
and were carried out at higher ionic strength. Hence these values are difficult to compare to those obtained from our direct observation of 1D motion along DNA at low salt concentration.

**Numerical simulations**

In order to account for the observed jump frequency and length distributions, we performed numerical Monte-Carlo simulations based on a combination of sliding (1D diffusion along the DNA) and jumping (3D diffusion) (details are given in Supplementary Data). We focussed on experimental data obtained at 10 mM [NaCl] for comparison with numerical results. Accordingly, we simulated the trajectory of an enzyme able to slide along a static, straight DNA molecule of finite length with a diffusion constant \( D_1 = 10^{-2} \mu m^2/s \). During its sliding motion, the enzyme could dissociate from the DNA. After dissociation, we simulated its 3D trajectory until a potential encounter with the DNA molecule. Upon the encounter, the protein re-associates to the DNA with a finite probability \( P \). After each encounter, the enzyme could resume its 3D diffusion with a probability \( 1 - P \). The surface onto which the DNA was attached was included in the simulations: during 3D diffusion, the enzyme could be reflected by an infinite plane located 70 nm below the DNA, this distance corresponding to the mean amplitude of the thermal transverse DNA fluctuations. A simulation was stopped when no encounter between the DNA and the enzyme occurred after a 3D walk of 40 ms. The photo-bleaching of the dye was also taken into account in the simulations (Supplementary Data). Ten thousand interaction events were simulated with enzyme starting points uniformly distributed along the DNA. Only simulated trajectories longer than 0.6 s were considered for generating jump length cumulative distributions in order to be consistent with our experimental results. The only adjustable parameter in our simulations is the probability \( P \) of binding to the DNA upon encounter. With decreasing \( P \), the number of large jumps decreases monotonously, and the jump length distribution derived from the numerical simulations with \( P = 0.02 \) is in excellent agreement with the experimental PIPES data (Figure 4C). For the other buffers the agreement is only qualitative, and, for instance, the number of large jumps observed in Tris remains slightly larger than predicted by the simulations, even with \( P = 1 \) (Figure 4C). Regarding the simplicity of our model, which does not include, for instance, the DNA fluctuations or the electrostatic interactions between the protein and the DNA, the fact that the probability of observing large jumps derived from our experiments can be qualitatively accounted for by our simulations is a further support for the conclusion that large jumps reported here stem from 3D translocations on the DNA.

**Interplay of 1D and 3D diffusion**

We have considered so far only large jumps, i.e. translocations larger than 200 nm. However, 3D excursions between two DNA sites separated by less than 200 nm are likely to occur. These small jumps are difficult to distinguish from sliding by optical means. According to the simulations, about one small jump occurred per interaction event in PIPES (Figure 4C). Moreover, the significantly higher number of large jumps observed in Tris together with the simulation results for \( P = 1 \) suggest that the number of invisible small jumps per interaction event might be up to a few tens per interaction event, depending on the experimental conditions. Although these values are rough estimations regarding the simplicity of the model, they are in qualitative agreement with previous theoretical works which predicted that small jumps occur more frequently than large-scale jumps (4,26,48).

The existence of small jumps might affect the estimation of the diffusion constant \( D_1 \), since what we have considered so far as a 1D motion (i.e. an interaction event without large jumps) reflects in fact the combination of sliding phases and small jumps. These small invisible jumps can lead to an ‘apparent’ 1D diffusion constant larger than that expected from pure sliding, because enzyme motion along DNA may be much faster when performed by a 3D than by a 1D mechanism. The effect of small jumps on the apparent diffusion constant, however, is significant only if the distance covered by sliding is comparable or smaller than that covered by 3D translocations during an interaction event. With increasing [NaCl], we observed a strong reduction (15-fold) of the interaction time while the jump distribution was almost maintained. Therefore, the duration of the sliding phases was strongly reduced, whereas the number of small jumps was not significantly altered. As a consequence, the distance covered by sliding was strongly reduced, whereas that covered by jumping remained almost unchanged, resulting in an increase of the apparent diffusion constant. Importantly, the diffusion coefficient does not change significantly for [NaCl] between 10 and 20 mM (Figure 5C), which confirms that our measurement of \( D_1 \) performed at low [NaCl] is not affected by small jumps.

A significant number of small jumps per interaction event can reconcile ensemble measurements that provided very different estimations for the sliding length, i.e. the DNA length explored by the enzyme during a sliding phase. This length was inferred to be larger than 1000 bp in studies where the length of non-specific DNA flanking an EcoRV recognition site was varied (30), whereas it was found to be below 100 bp in experiments involving DNA with two recognition sites in close proximity (34). This discrepancy could be explained by considering that, due to hopping/jumping, the DNA length effectively explored by the protein after an initial binding to the nucleic acid could be much larger than the sliding length itself. Finally, we point out that the observed jump distributions were measured on an elongated substrate, whereas free DNA in solution adopts a coiled conformation. For small jumps, elongated and coiled conformations should be comparable as the DNA is expected to be rigid for lengths smaller than the DNA persistence length (50 nm). The coiled conformation, however, considerably favours large jumps, which can not only occur onto DNA sequences nearby, but also to distant sequences which nevertheless are close in space (24). Therefore, it is likely that the average number of jumps per interaction
event is significantly larger for coiled DNA than for elongated DNA (38).

In summary, we report here the first direct observation of sliding and jumping for individual EcoRV enzymes interacting with nonspecific DNA. Our investigation regarding both 1D Brownian motion (sliding) and 3D translocations (jumping), combined with numerical simulations and salt-dependent experiments, allowed us to quantitatively estimate the respective part of these two processes in the facilitated diffusion of EcoRV under our experimental conditions. As far as we know, a jumping process has never been directly observed earlier with any DNA-binding protein, although it was postulated in many previously published experimental and theoretical studies. The contribution of hopping/jumping to facilitated diffusion, however, might differ from protein to protein, depending on both the structure and the biological function of the protein.

The small jumps that are inferred from our study can answer the problem of target search within a crowded environment (20) as they enable the enzyme to bypass obstacles of typical protein size that could block sliding. On the other hand, large jumps favour the exploration of distant DNA sites, especially when the DNA is in a coiled conformation, thus accelerating target site location compared to sliding alone.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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Supplementary Data.

MSD calculation. The MSD $g(\tau)$ of a variable $x(t)$ is a statistical function defined by

$$g(\tau) = \langle [x(t+\tau) - x(t)]^2 \rangle,$$

where $\langle \rangle$ denotes an average over time. Practically, the MSD for the time interval $n\Delta T$ was estimated using:

$$MSD = \frac{1}{N} \sum_{i=1}^{N} (x_{i+n} - x_i)^2/(N-n),$$

where $\Delta T$ is the exposure time (20 ms), $N$ the total number of frames of the movie and, for the frame $i$, $x_i$ denotes the position of the enzyme along the DNA (for the longitudinal MSD) or perpendicular to the DNA (for the transverse MSD). The error bars in the MSD($n\Delta T$) curves corresponding to single enzyme trajectories were calculated using a statistical approach (1). The slope of the longitudinal MSD($n\Delta T$) curves was obtained by least-square fitting between 20 and 100 ms (1 to 5 video frames)(2). The diffusion constant $D_1$ was deduced from this slope by dividing it by twice the square of the DNA stretch rate (ratio of the end-to-end length of the elongated DNA, measured for each molecule, to its contour length). The stretch rate must be included in the calculation of $D_1$ for taking into account the effective translocation of the enzyme along the DNA (i.e., the measured translocation divided by the DNA stretch rate) during sliding.

In order to circumvent the statistical variance inherent to the derivation of $D_1$ from individual enzyme trajectories, we averaged the MSD($n\Delta T$) plots derived from all the events recorded under the same experimental conditions. Before averaging, the MSD($n\Delta T$) plots were corrected for the DNA stretch rate, which could slightly change from DNA to DNA. The main error in the determination of $D_1$ stems from the error in the evaluation of the stretch rate. This error is due to the inaccuracy of the localization of the DNA ends, obtained from the fluorescence image of the DNA after SybrGold staining. Assuming a localization accuracy of one pixel (~120 nm) for the DNA end-to-end distance (~2 μm), we obtained $\Delta D_1 \approx 0.2 D_1$.

DNA thermal fluctuations. In order to estimate the effect of the thermal fluctuations of the DNA on the MSD($n\Delta T$) plots, we calculated the amplitudes $\langle A_t^2 \rangle$ and $\langle A_l^2 \rangle$ and the correlation times $\tau_t$ and $\tau_l$ associated with the transverse and longitudinal DNA fluctuations using a “bead and spring” model (3). Averaged over an elongated DNA molecule of contour length $L$, the amplitudes $\langle A_t^2 \rangle$ and $\langle A_l^2 \rangle$ are given by (4):

$$\langle A_t^2 \rangle = \frac{L^2 R}{9f_t (R/L)}$$

and

$$\langle A_l^2 \rangle = \frac{L^2 R}{9f_l (R/L)},$$

where $R$ is the end-to-end distance of the elongated DNA molecule, $L_p$ is the persistence length of the DNA (50 nm), and the functions $f_t$ and $f_l$ account for the nonlinear elasticity of the DNA (5,6):

$$f_t (R/L) = \frac{2}{3} \frac{L}{6R} \left( 1 - \frac{1}{1-R/L} \right)^{-1}$$

and

$$f_l (R/L) = \frac{2}{3} \frac{1}{3(1-R/L)^2}.$$

We used a T7 DNA fragment of length $L=3$ μm, which was elongated to $R/L \approx 0.7$. These values lead to $\langle A_t^2 \rangle \approx (70 \text{ nm})^2$ and $\langle A_l^2 \rangle \approx (35 \text{ nm})^2$. The transverse and longitudinal correlation times $\tau_t$ and $\tau_l$ associated with the first normal modes of the DNA fluctuations are given by (4,6):

$$\tau_t = \frac{8\eta L^2 R}{3\pi \ln(L/d) k_B T f_t (R/L)}$$

and

$$\tau_l = \frac{4\eta L^2 R}{3\pi \ln(L/d) k_B T f_l (R/L)},$$

where $d$ is the diameter of the DNA (2 nm) and $\eta$ is the water viscosity ($10^{-3}$ Pa.s). In our experiments, $\tau_t \approx 5$ ms and $\tau_l \approx 1$ ms. A straightforward calculation shows that the contributions $g_{ij}^{DNA}(\tau)$ of the DNA fluctuations to the transverse and longitudinal MSD($n\Delta T$) plots are given by:

$$g_{ij}^{DNA}(n\Delta T) = 2 \langle A_i^2 \rangle \left( 1 - \exp(-n\Delta T/\tau_i) \right).$$

Since $\tau_t$ and $\tau_l$ are much smaller than the...
exposure time ($\delta T = 20$ ms), the function $g^{\text{DNA}}_{ij}(n\delta T)$ reduces to $2\langle A^2 \rangle$. Thus, the fluctuations of the DNA contribute as an offset to the transverse and longitudinal MSD($n\delta T$) plots. Yet these offsets in the MSD($n\delta T$) plots presented here are smaller than those expected from the above estimations of $\langle A^2 \rangle$ and $\langle A^2 \rangle$, because the position of the proteins was averaged over a substantial number of DNA fluctuations during the acquisition time.

**Localization accuracy.** In order to estimate the localization accuracy in our experiments, we calculated both the longitudinal and transverse MSD $g^{\text{spot}_{n\delta T}}_{ij}$ of fluorescently-labeled proteins that occasionally attached to the surface. Since the uncertainty in the measurement of the enzyme position is a random error, i.e., an error that is not correlated with time, are expected to be constant (1): $g^{\text{spot}_{n\delta T}}_{ij} \approx 2\sigma_{ij}^2$, where $\sigma_{ij}$ are the standard deviations of the distributions of the transverse and longitudinal positions of the enzyme. The averaged MSD($n\delta T$) plots derived from the analysis of 112 proteins fixed to the surface are in excellent agreement with this prediction (Figure S1 (a)). We deduced from these curves that $2\sigma_{ij}^2 \approx 10^{-3}$ $\mu$m$^2$, which leads to $\sigma_{ij} \approx 22$ nm. Yet the localization accuracy $\sigma$ for enzymes interacting with the DNA was re-evaluated, about 30 nm, as the fluorescence signal for these enzymes was about half of that of surface-attached enzymes. This decrease of the fluorescence signal results from a weaker excitation of the labeled enzymes due to the fast decay of the evanescent wave intensity with distance from the surface.

**Discrimination between surface-attached enzymes and enzymes interacting with the DNA.** The MSD calculation can be used not only to estimate the diffusion constant $D_1$, but also to discriminate between the enzymes interacting with DNA and those stuck to the surface. Indeed, the MSD($n\delta T$) plots for both the longitudinal and transverse motion of an enzyme interacting with the DNA display additional offsets compared to the MSD($n\delta T$) plots for a surface-immobilized enzyme. These offsets are due to the thermal fluctuations of the DNA, which superimpose with the motion of an enzyme diffusing along the DNA (Figure S1 (a): averaged MSD, Figure S1 (a-f): MSD calculated from individual events). Notably, since the amplitude of the transverse DNA fluctuations is twice that of the longitudinal ones (see above), the offset for the transverse MSD is larger than that for the longitudinal MSD. Overall, for an enzyme interacting with the DNA, the value of the transverse MSD is typically more than $2 \times 10^{-3}$ $\mu$m$^2$ (Figure S1(b-d)), compared to the value of the transverse MSD for a protein stuck to the surface, which is typically below $10^{-3}$ $\mu$m$^2$ (Figure S1(e-g)). Therefore, even in cases where the linear dependence on time of the longitudinal MSD is difficult to distinguish due to statistical noise, a large amplitude of the transverse MSD is a good indication that the tracked enzyme was interacting with the DNA.

**Probability of large steps by sliding.** The mean length $l$ of a 1D Brownian motion step during $\Delta T = 2\delta t$ is given by $l = 2D_1\Delta T$, where $D_1$ is the diffusion constant. For $D_1 = 10^{-2}$ $\mu$m$^2$/s and $\Delta T = 40$ ms, we get $l \approx 30$ nm. This length is also the standard deviation $\mu$ of the length distribution of the $N = T/2\Delta T$ steps that occurs during an interaction event of duration $T$. The expected number of steps whose length is larger than 200 nm = 7 $\mu$ is given by $N \text{erfc}(7/\sqrt{2}) = 10^{-12}$ $N$ (erfc(x)) is the complementary error function). Since all the analyzed sliding data comprises about $N = 10^8$ frames, the probability of observing even one step larger than 200 nm is then extremely low.

**Elimination of alternative explanations for large jumps.** The possibility that the large jumps observed during our experimental records do not stem from a fast translocation of the same enzyme along the DNA has been considered. For example, a second enzyme may bind to the DNA immediately after a first enzyme dissociated at a different position. The probability of such an event
can be estimated from the mean duration \( n \) of the interaction events and the mean time lapse \( N \) between two consecutive interaction events (\( n \) and \( N \) denote the respective number of frames). Within a time span of \( 2(N+n) \), on average two events of duration \( n \) are detected. Considering the limitation that an interaction event must start after the first frame and end before \( 2(N+n) \) frames, \( (2N+n)^2 \) event combinations are possible. Among these combinations, about \( 2\cdot(2N+n) \) combinations relate to an event that ends within two frames before another one begins. Hence, the probability \( p \) of a false jump is \( p \approx 4/(2N+n) \), so with \( N = 300 \) (i.e., 6s) and \( n = 50 \) (i.e., 1s) under typical experimental conditions, this gives \( p < 0.5 \% \). It may also be possible that enzymes jump from or to the surface to which the DNA is attached. As explained above, the MSD(\( n\delta T \)) plots derived from the trajectory of the enzyme allowed us to detect such events, which occurred only rarely and were not taken into account as large jumps.

**Experiment under flow.** We designed a flow cell in order to apply a flow with perpendicular orientation to the stretched DNA (Figure S2 (a)). Briefly, an additional hole pair was drilled into the microscope slide, and the parafilm sealing was cut in a cross shape to incorporate the four holes. Two adjacent ports of the slide (III and IV) were connected to the syringe pump using a valve that allowed us to switch from one channel system to the other. The DNA was stretched by application of a flow in one direction - inlet port I, outlet port III-, while the inlet port II was sealed. After DNA stretching, the valve was switched to the outlet port IV and a flow was applied in the perpendicular direction –inlet port II, outlet port IV-. We used the DNA bending due to the flow to estimate the velocity \( v \) of the flow in the DNA region (Figure S2 (b-c)). We assumed that the flow exerted on the DNA a homogeneous force proportional to both \( v \) and the viscosity \( \eta \) of the fluid, balanced by the nonlinear DNA entropic force: \( T(R)=k_0 f_i (R/L)R \), where \( k_0=\frac{3k_BT}{2L^2\eta} \). Assuming that the maximal deviation \( d \) of the DNA is small compared to the end-to-end distance \( R \), the velocity \( v \) is given by:
\[
v=\frac{8k_0 f_i (R/L)}{\eta R} \ d \ .
\]
We measured \( d \approx 150 \) nm, which yields \( v \approx 70 \) \( \mu \)m/s.

**Estimation of the photobleaching time constant.** Due to the photobleaching of the dye, the mean duration \( T_i \) of the interaction events that we recorded can be smaller than the mean interaction time \( T \) the enzyme spent interacting with the DNA. In order to address this effect, we estimated the photobleaching time constant \( T_p \) by determination and comparison of \( T_i \) and \( T \) in the different buffers. We calculated the duration of each interaction event by considering the number of frames that composed individual trajectories. The complementary cumulative distributions of the durations in different buffers are displayed in Figure S3A. Monoexponential fits of the distributions provided the mean duration \( T_i \) in the different buffers: \( T_i=1.1\pm0.5 \) s in HEPES, \( T_i=1.3\pm0.5 \) s in PIPES, \( T_i=1.5\pm0.5 \) s in PB, and \( T_i=1.6\pm0.5 \) s in Tris. In order to estimate \( T \), we circumvented photobleaching by recording events at low laser intensity (8 W/cm\(^2\)), with the drawback that the exposure time had to be increased to 300 ms for enzyme detection. Although the corresponding trajectories were not suitable for analyzing sliding or large jumps, we assumed that the event durations could be used for the determination of \( T \). For each buffer, we recorded about 100 interaction events, and the complementary cumulative distributions of the event durations were fitted with monoexponential functions (Figure S3B). Interaction times \( T \) (\( T=2.1\pm0.5 \) s in HEPES, \( T=4.4\pm1 \) s in PIPES, \( T=7.2\pm1.5 \) s in PB, and \( T=9.1\pm2.0 \) s in Tris) are larger than \( T_i \), confirming that the trajectories recorded under high laser intensity were shortened by photobleaching. The photobleaching time \( T_p \) was evaluated assuming that, at high laser intensity, the photobleaching rate combined with the enzyme dissociation rate. This hypothesis yields \( 1/T_i = 1/T_p + 1/T_p \), i.e., \( T_p=T_i/(T-T_i) \), which gives approximately the same value for the four buffers considered: \( T_p \approx 2.0 \) s (\( T_p \approx 2.3 \) s in HEPES, \( T_p \approx 1.9 \) s in PIPES , \( T_p \approx 1.9 \) s in PB, and \( T_p \approx 2.0 \) s in Tris).
Expected effects of the salt concentration on sliding and jumping. Since non-specific interactions between DNA and proteins are mainly driven by electrostatics, they are strongly affected by changes in salt concentration. Kinetically, these interactions are described by the non-specific association rate \( k_a \) and the non-specific dissociation rate \( k_d \). The effect of changes of salt concentration is different depending on whether one considers \( k_a \) or \( k_d \). Because of entropic reasons, \( k_d \) is strongly dependent on ionic conditions and it decreases significantly with increasing salt concentration (7,9). On the other hand, \( k_a \) depends on the screening effect of cations around the negatively charged backbone of the DNA. The latter is known to only moderately decrease with increasing monovalent ion concentration in the presence of divalent ions, such as Mg\(^{2+}\) (7,9-11).

The rates \( k_a \) and \( k_d \) can be connected to sliding and jumping: \( k_d \) is linked to the dissociation probability of a DNA-bound enzyme, and thus determines the average duration of a “pure” sliding event, i.e., without jumps, while \( k_a \) determines the probability of binding to the DNA, and thus is connected to jumping. Therefore, the main effect of increasing [NaCl] is to shorten the duration of sliding interactions while the number of jumps should decrease only moderately. The relative importance of jumping to the facilitated diffusion of an enzyme is increased at high [NaCl] since the length covered by 3D jumping changes only slightly while the length explored by sliding decreases drastically.

Sliding and jumping of EcoRV at different salt concentrations. Salt-dependent experiments were carried out in PIPES. The measurement of the diffusion constant at different salt concentrations was carried out in a similar way to the determination of \( D_1 \) at low salt. All events longer than five frames were considered in order to overcome the large decrease of the interaction time with increasing salt. The highest [NaCl] considered was 60 mM, because at higher concentrations the interaction time was too short for a reliable estimation of \( D_1 \). Translocations over more than 200 nm within two frames were associated with large jumps, and quantified using complementary cumulative distributions. All curves were normalized with respect to the data obtained at the lowest salt concentration in PIPES, i.e., 10 mM NaCl. As explained above, the mean interaction time \( T \) at 10 mM NaCl \((T = 4.4\pm1.0 \text{ s in PIPES})\) was estimated at low laser intensity (8 W/cm\(^2\), 300 ms CCD exposure time) in order to circumvent photobleaching. With increasing salt concentration, \( T \) strongly decreases, and we had to decrease the CCD exposure time in order to get a number of frames per interaction event sufficient to reliably estimate \( T \). In parallel, we slightly increased the laser intensity in order to get enough fluorescence signal. Besides, since at high [NaCl] \( T \) became much smaller than the photobleaching time constant \((T_p \sim 2.0 \text{ s at high laser intensity, i.e., 100 W/cm}^2\), see above), the measurement of \( T \) was only slightly affected by the photobleaching of the dye. The complementary cumulative distributions of the durations at different salt concentrations are displayed in Figure S4. Monoexponential fits of the distributions provided the mean duration \( T \) at different [NaCl]: \( T = 2.7 \pm0.6 \text{ s at 20 mM NaCl (laser intensity 15 W/cm}^2\), CCD exposure time 200 ms), \( T = 0.8 \pm0.2 \text{ s at 40 mM NaCl (laser intensity 25 W/cm}^2\), CCD exposure time 100 ms), \( T = 0.3 \pm0.1 \text{ s at 60 mM NaCl (laser intensity 100 W/cm}^2\), CCD exposure time 20 ms).

FCS experiments. The hydrodynamic radius of fluorescently-labeled enzymes was measured on a home built two-photon set-up(12). Fluorescence was excited with a mode-locked titanium-sapphire laser (Mira pumped by Verdi, Coherent). Fluorescence photons were collected with an Olympus UplanApo 60X 1.2 NA water immersion objective through filters (AHF Analysentechnik) and optical fibers (FG200LCR multimode fiber, Thorlabs) connected to two avalanche photo-diodes (SPCM-AQR-14, Perkin Elmer, Vaudreuil, Canada) coupled to an ALV-6000 correlator (ALV GmbH). The excitation input power was determined by a Lasermate powermeter (Coherent) or a Nova II powermeter (Ophir Optronics Ltd.). Powers were kept under 5 mW in order to stay in the
range of quadratic absorption dependence. The intensity and the temporal correlation function \( G(\tau) \) of the collected fluorescence emission were recorded. The following equation was used to fit the experimental FCS autocorrelation curves (13): \( G(\tau) = \frac{1}{N} \frac{1}{1 + \tau/\tau_D} \), where \( N \) is the average number of molecules in the illuminated spot, described as a 2D Gaussian, and \( \tau_D \) is the diffusion time of the molecules through the beam waist \( \omega = 0.3 \, \mu m \). Under two-photon excitation \( \tau_D \) depends on the beam waist \( \omega_{xy} \) and on the diffusion coefficient \( D_3 \) as \( \tau_D = \frac{\omega_{xy}^2}{8D_3} \) (14). The hydrodynamic radius \( r_H \) is then given by the Stokes-Einstein relation \( D_3 = \frac{k_B T}{6 \pi \eta r_H} \), where \( \eta \) is the viscosity of the buffer at temperature \( T \) (10^7 Pa.s) and \( k_B \) the Boltzmann constant.

Monte-Carlo numerical simulations. We performed numerical simulations to estimate the respective contributions of sliding (1D diffusion) and jumping (3D diffusion). The space was subdivided into cubic cells of size \( d = 0.34 \, \text{nm} \), i.e., the distance between two adjacent DNA bps, for the simulation of 1D and 3D motion. The DNA was assumed to be a straight cylinder of length \( L = 2 \, \mu m \) and radius \( R_{\text{DNA}} = 1 \, \text{nm} \) and the enzyme to be a sphere of radius \( R_{\text{enz}} = 4 \, \text{nm} \). For simplification, we used in the simulations a point-like sphere and a DNA of radius \( a = R_{\text{DNA}} + R_{\text{enz}} \), both models being mathematically identical.

For simulation of the diffusion processes, the enzyme motion was likewise subdivided into steps, i.e., motion from a cell to one of the neighboring cells. The times \( \tau_1 \) and \( \tau_2 \) required for such a step by, respectively, 1D and 3D diffusion were calculated from the theoretical Brownian motion: \( \tau_1 = d^2/(2D_1) \) and \( \tau_3 = d^2/(6D_3) \). The diffusion constants \( D_1 \) and \( D_3 \) were respectively taken from our single-molecule measurements (i.e., \( D_1 = 10^{-2} \mu m^2/s \)) and FCS experiments (\( D_3 = 50 \mu m^2/s \)). A simulation started with the enzyme positioned anywhere on the DNA and sliding. DNA ends were considered to be reflective, i.e., whenever the point-like sphere reached one of the two ends of the cylinder along the DNA axis, it could only move backwards. Before each 1D step, the enzyme had a small probability \( \varepsilon \) of dissociating from the DNA, in which case it was placed in the cell outside of the cylinder adjacent to its previous position. The probability \( \varepsilon \) was adjusted so that the average duration of the simulation events matched the DNA-EcoRV interaction time \( T \) estimated in experiments at low laser intensity (e.g., \( T \sim 4.4 \, s \) in PIPES). In the case that the enzyme dissociated from the DNA, 3D diffusion was simulated by moving the point-like sphere to one of the six neighboring cells with equal probability. An encounter of free enzyme and DNA occurred whenever the point-like sphere entered a cell position within the DNA cylinder during the 3D walk. Upon collision, the enzyme had a probability \( p \) of binding to the DNA and to start again a sliding motion. Otherwise, i.e., with a probability \( 1-p \), the 3D random walk resumed. The surface onto which the DNA was attached was included in the simulations: when the point-like sphere collided with an infinite plane located 70 nm below the DNA, it was reflected. The simulation of each trajectory ended when the enzyme did not rebind to the DNA within 40 ms of 3D walk. The trajectory of the enzyme was then reconstructed by averaging the position of the enzyme over successive sets of 20 ms (i.e., CCD exposure time). The number of simulated trajectories was \( N_s = 10000 \).

In order to take into account photobleaching of the dye, we generated a set of \( N_s \) exponentially distributed random times, with a mean value corresponding to the photobleaching time constant \( T_p \) (\( T_p \sim 2.0 \, s \)). The duration of the \( i^{\text{th}} \) (\( 1 \leq i \leq N_s \)) trajectory was then compared with the \( i^{\text{th}} \) random time. In case of duration larger than the random time, the length of the trajectory was shortened to the value of the random time. We checked that the mean duration of the \( N_s \) simulated trajectories was reduced to the mean duration of the experimental interaction events \( T_i \) (e.g., \( T_i \sim 1.3 \, s \) for PIPES). Finally, for comparison with our experimental results, we rejected the simulated trajectories shorter than 30 frames (i.e., 600 ms).
Single molecule experiments with Atto647N-labeled EcoRV. To verify that labeling with Cy3B has no influence on the enzyme dynamics, experiments were performed with an Atto647N-labeled protein. For labeling, 180 pmoles of EcoRV mutant in 35 µl PBS was added to 1.85 µl (10 mM) of Atto647N maleimide (Sigma) in DMSO and 1.3 µl of triscarboxyethyl-phosphine (TCEP, 100mM in PBS). After incubation for 2 h on ice, the reaction was stopped by addition of BME. The mixture was first purified on a Ni-column (Protino 500) by washing with PBS, PBS containing 1 M NaCl and a solution of 1 M NaCl with 1 % Triton X100. Elution of the labeled enzyme was preceded by an elution buffer containing 200 mM imidazol, followed by a second purification on a heparin column. After elution from the heparin column with TE buffer containing 600 mM NaCl, the product was finally dialyzed against TE buffer with 300 mM NaCl, 1 mM DTT and 50 % glycerol. About 85 % of the proteins were recovered with a labeling ratio of better than 1.6 Atto647N-dye per EcoRV. The activity of the labeled enzyme was checked using a standard DNA cleavage assay. Single-molecule experiments were carried out in PB buffer, and 55 interaction events of Atto647N-labeled EcoRV with elongated DNA were analyzed. The results regarding both the diffusion constant $D_1$ and the jump length distributions are similar to those obtained with Cy3B-labeled EcoRV in the same buffer (Figure S5).

Figure S1: Comparison between the MSD of enzymes interacting with the DNA and the MSD of enzymes stuck to the surface. (a) The longitudinal (●) and transverse (■) MSD averaged over 102 DNA/enzyme interaction events in PIPES display additional offsets compared to the longitudinal (▲) and transverse (▼) MSD calculated from enzymes attached to the surface (MSD averaged over 112 static spots). These additional offsets are due to the DNA thermal fluctuations and can be used to discriminate the enzymes interacting with the DNA from those bound to the surface. (b-d) Transverse (■) and longitudinal (▲) MSD of individual enzymes stuck to the surface ((b): 37 frames, (c): 62 frames, (d) 93 frames). (e-g) Transverse (■) and longitudinal (●) MSD of individual enzymes interacting with the elongated DNA ((e): 36 frames, (f): 53 frames, (g): 88 frames). The linear time dependence of the longitudinal MSD and the large amplitude of the transverse MSD combine to prove that the enzymes were interacting with the DNA.
Figure S2: Interaction of EcoRV with DNA under perpendicular flow. (a) The flow cell was modified to apply a perpendicular flow: ports I and II were connected to the syringe pump via an adjustable valve while ports I and III were alternatively sealed or connected to the reservoir. For DNA stretching the ports I/III were used, whereas for the measurement of interaction under flow the system was switched to ports II/IV. (b) Fluorescence image of stained DNA (SybrGold). The molecule is stretched to about 70% of its contour length. (c) The application of a flow of ≈ 200µm/s leads to a significant bending of the DNA molecule, whose center is displaced by about 400 nm with respect to the line defined by the ends of the DNA.

Figure S3: Estimation of the photobleaching time constant (a) Complementary cumulative distributions of the durations of the interaction events in different buffers under conditions used for the investigation of the facilitated diffusion of EcoRV. (b) Complementary cumulative distributions of the interaction times in different buffers measured at low laser intensity.

Figure S4: Complementary cumulative distributions of the durations of the interaction events in PIPES buffer at different salt concentrations (10 to 60 mM NaCl). At low salt, the laser intensity was reduced to circumvent the dye photobleaching of the dye.
Figure S5: Comparison of single molecule results obtained with Cy3B- and Atto647N-labeled EcoRV. Both the $D_1$-distribution (a) and the jump length cumulative (b) are similar for the two differently labeled enzymes.

Bibliography