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 \( \langle \cdot \rangle \) denotes an average over time. Practically, the MSD for the time interval \( n\Delta T \) was estimated using:
\[
MSD(n\delta T) = \frac{\sum_{i=1}^{N} (x_{i+n} - x_i)^2}{(N-n)},
\]
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 \( \mu \)m), 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 \( <A_t^2> \) and \( <A_l^2> \) 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 \( <A_t^2> \) and \( <A_l^2> \) are given by (4):
\[
<A_t^2> = \frac{L_{p}^2}{9f_t(R/L)} \quad \text{and} \quad <A_l^2> = \frac{L_{p}^2}{9f_l(R/L)},
\]
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):
\[
f_t(R/L) = \frac{2}{3} \frac{L}{6R} \left( \frac{1}{1-R/L} \right)^{-1} \quad \text{and} \quad f_l(R/L) = \frac{2}{3} \frac{1}{3(1-R/L)^2}.
\]
We used a T7 DNA fragment of length \( L=3 \mu \)m, which was elongated to \( R/L \approx 0.7 \). These values lead to \( <A_t^2> \approx (70 \text{ nm})^2 \) and \( <A_l^2> \approx (35 \text{ nm})^2 \). 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):
\[
\tau_t = \frac{8\eta L_{p}^2}{3\pi ln(L/d)k_BT f_t(R/L)} \quad \text{and} \quad \tau_l = \frac{4\eta L_{p}^2}{3\pi ln(L/d)k_BT f_l(R/L)}
\]
where \( d \) is the diameter of the DNA (2 nm) and \( \eta \) is the water viscosity (10\(^3\) Pa.s). In our experiments, \( \tau_t \approx 5 \text{ ms} \) and \( \tau_l \approx 1 \text{ ms} \). A straightforward calculation shows that the contributions \( g^{(\text{DNA})}_{t/l}(\tau) \) of the DNA fluctuations to the transverse and longitudinal MSD(\( n\delta T \)) plots are given by:
\[
g^{(\text{DNA})}_{t/l}(n\delta T) = 2(\mathcal{A}_t/l)\left(1-\exp(-n\delta T/\tau_{t/l})\right).
\]
Since \( \tau_t \) and \( \tau_l \) are much smaller than the
exposure time \((\delta T = 20 \text{ ms})\), the function \(g_{1T}^{\text{MN}}(n\delta T)\) reduces to \(2\langle A_{1}^{2} \rangle\). 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 \(<A^{2}_{T}>\) and \(<A^{2}_{L}>\), 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 \(g_{1T}^{\text{apo}}(n\delta T)\) 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, \(\sigma_{i,j}\) are expected to be constant (1): \(g_{1T}^{\text{apo}}(n\delta T)\approx2\sigma_{i,j}^{2}\), where \(\sigma_{i,j}\) 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 \(2\sigma_{i,j}^{2}\approx10^{-3} \text{ \mu m}^{2}\), which leads to \(\sigma_{i,j}\approx22 \text{ nm}\). 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 \(D_{i}\), 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 of the longitudinal MSD. Overall, for an enzyme interacting with the DNA, the value of the transverse MSD is typically more than \(2 \times 10^{-3} \text{ \mu m}^{2}\) (Figure S1(b-d)), compared to the value of the transverse MSD for a protein stuck to the surface, which is typically below \(10^{-3} \text{ \mu m}^{2}\) (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 \(P = 2D_{T}\Delta T\), where \(D_{T}\) is the diffusion constant. For \(D_{T} = 10^{-2} \text{ \mu m}^{2}\text{s}\) and \(\Delta T = 40 \text{ ms}\), we get \(l = 30 \text{ nm}\). 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 \(200 \text{ nm} = 7 \mu\) is given by \(N \text{erfc}(7/\sqrt{2}) = 10^{-12} N\) \((\text{erfc}(x))\) is the complementary error function). Since all the analyzed sliding data comprises about \(N = 10^{6}\) 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 events 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 stretching, the valve was switched to the outlet port IV and a flow was applied in the 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: \( T(R)=k_o f_i (R/L) R \), where \( k_o=\frac{3k_BT}{2L^2} \). 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:

\[
 v=\frac{8k_o f_i (R/L)}{\eta R} d
\]

We measured \( d \approx 150 \text{ nm} \), which yields \( v \approx 70 \mu \text{m/s} \).

**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: \( T_i=1.1\pm0.5 \text{ s} \) in HEPES, \( T_i=1.3\pm0.5 \text{ s} \) in PIPES, \( T_i=1.5\pm0.5 \text{ s} \) in PB, and \( T_i=1.6\pm0.5 \text{ s} \) 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 \( T (T=2.1\pm0.5 \text{ s} \text{ in HEPES, } T=4.4\pm1 \text{ s} \text{ in PIPES, } T=7.2\pm1.5 \text{ in PB, and } T=9.1\pm2.0 \text{ in Tris}) \) 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 \( 1/T_i = 1/T + 1/T_p \), i.e., \( T_p=T/((T-T_i)) \), which gives approximately the same value for the four buffers considered: \( T_p \approx 2.0 \text{ s} \text{ (Hepes, } T_p \approx 1.9 \text{ s in PIPES, } T_p \approx 1.9 \text{ s in PB, and } T_p \approx 2.0 \text{ s in Tris}) \).
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 $k_d$ (7,8). 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 ($T = 4.4 \pm 1.0$ 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 ($T_p \sim 2.0$ 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]: $T = 2.7 \pm 0.6$ s at 20 mM NaCl (laser intensity 15 W/cm$^2$, CCD exposure time 200 ms), $T = 0.8 \pm 0.2$ s at 40 mM NaCl (laser intensity 25 W/cm$^2$, CCD exposure time 100 ms), $T = 0.3 \pm 0.1$ 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 $\tau_D = \omega_{xy}^2 / 8 D_3$ (14). The hydrodynamic radius $r_H$ is then given by the Stokes-Einstein relation $D = k_B T / 6 \pi \eta r_H$, where $\eta$ is the viscosity of the buffer at temperature $T$ ($10^{-3}$ 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 $a = 0.34 \, nm$, 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 $R_{DNA} = 1 \, nm$ and the enzyme to be a sphere of radius $R_{enz} = 4 \, nm$. For simplification, we used in the simulations a point-like sphere and a DNA of radius $a = R_{DNA} + R_{enz}$, 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: $\tau_1 = d^2 / (2 D_1)$ and $\tau_2 = d^2 / (6 D_3)$. 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 cold 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 reached one of the two ends of the cylinder along the DNA axis, it cold 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 $i^{th}$ ($1 \leq i \leq N_s$) trajectory was then compared with the $i^{th}$ 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 pmol 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.](image-url)
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 \(\approx 200\mu\text{m/s}\) 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.

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