Probing DNA conformational changes with high temporal resolution by tethered particle motion

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Abstract

The tethered particle motion (TPM) technique informs about conformational changes of DNA molecules, e.g. upon looping or interaction with proteins, by tracking the Brownian motion of a particle probe tethered to a surface by a single DNA molecule and detecting changes of its amplitude of movement. We discuss in this context the time resolution of TPM, which strongly depends on the particle–DNA complex relaxation time, i.e. the characteristic time it takes to explore its configuration space by diffusion. By comparing theory, simulations and experiments, we propose a calibration of TPM at the dynamical level: we analyze how the relaxation time grows with both DNA contour length (from 401 to 2080 base pairs) and particle radius (from 20 to 150 nm). Notably we demonstrate that, for a particle of radius 20 nm or less, the hydrodynamic friction induced by the particle and the surface does not significantly slow down the DNA. This enables us to determine the optimal time resolution of TPM in distinct experimental contexts which can be as short as 20 ms.

\[\text{Online supplementary data available from stacks.iop.org/PhysBio/7/046003/mmedia}\]

1. Introduction

Biophysical techniques at the single molecule level have become an integral part of the available toolbox to investigate biomolecular machineries. The rapid development of experimental techniques for the exploration of conformations and dynamics of single DNA molecules emphasize the need for suitable theoretical tools to interpret the large amount of data collected. Among the many techniques used in biology or biophysics laboratories, tethered particle motion (TPM) \cite{1,2,3,4,5,6,7,8,9,10,11,12,13,14} is very promising because it explores the \textit{equilibrium} statistical mechanics of the biopolymer in the absence of external force, by contrast to optical or magnetic tweezers experiments \cite{15}. One end of a DNA molecule is immobilized on a glass surface, and the other end is attached to a particle, the diameter of which ranges from a few tens to several hundreds of nanometers (figure 1). By measuring DNA end-to-end distance (or effective length), tracking by video microscopy the particle trajectory informs about DNA conformations in real time. Hence TPM also gives access to \textit{dynamical} properties. Getting insights into the dynamics of biomolecular events is of great significance. If its time resolution is sufficient,
changes induced by enzyme processing [1, 3]. Such sliding time intervals of a given duration, conformational changes are detected through variations of unbinding [4, 16], hybridization [4–7, 14], curvature variations due to protein binding.

Figure 1. Snapshot of TPM Monte Carlo simulation: the tethered DNA molecule is modeled as a coarse-grained chain of N connected spheres (various hues), fixed to the glass surface at one end and to the tracked particle at the other end. Here, N = 80, the DNA contour length is \( L = 2080 \) base pairs (\( \simeq 700 \) nm) and the particle radius is \( R = 150 \) nm.

TPM has the capability to get access to the dynamics of DNA conformational changes, such as looping/unlooping [4–7, 14], curvature variations due to protein binding/unbinding [4, 16], hybridization/dehybridization [17] or changes induced by enzyme processing [1, 3]. Such conformational changes are detected through variations of the particle amplitude of movement, which is calculated on sliding time intervals of a given duration, \( T_{av} \). On the one hand, \( T_{av} \) must be long enough to have a good estimate of the amplitude of movement, and thus to discriminate different amplitudes associated with different DNA conformations. On the other hand, \( T_{av} \) sets the TPM time resolution because events shorter than \( T_{av} \) are smeared out and thus cannot be detected. Therefore \( T_{av} \) must be optimally chosen [6]. We shall see below that its optimal value is proportional to the relaxation time of the particle–DNA complex, i.e. the characteristic time the complex takes to explore its configuration space by diffusion. Thus the knowledge of the relaxation time is a prerequisite to estimate the TPM time resolution.

The experimental conditions, especially the proximity of the surface and the attachment of the particle, are likely to perturb the polymer and to increase the relaxation time. Therefore knowing the relaxation time in the function of both the DNA contour length and the particle radius is of primary importance to anticipate TPM time resolution capabilities.

To our knowledge, dynamical consequences of the setup geometry, in particular of the attached particle, have not been quantified extensively yet. Calibration of TPM experiments, at the dynamical level, remains to be performed. We focus on the DNA relaxation time, technically defined as the characteristic time the complex takes to explore its configuration space. Knowing the relaxation time in the function of both the DNA geometry, in particular of the attached particle, have not been quantified extensively yet. Calibration of TPM experiments, at the dynamical level, remains to be performed. We focus on the DNA relaxation time, technically defined as the characteristic time the complex takes to explore its configuration space.

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To our knowledge, dynamical consequences of the setup geometry, in particular of the attached particle, have not been quantified extensively yet. Calibration of TPM experiments, at the dynamical level, remains to be performed. We focus on the DNA relaxation time, technically defined as the characteristic time the slowest elastic mode only. We analyze how it scales with particle radius and DNA length, by using small particles with radii down to 20 nm, for DNA molecules of length \( L \) ranging between 401 and 2080 base pairs (bp; 1 bp = 0.34 nm). The DNA is semi-flexible since \( L \) is on the order of the persistence length, \( \ell_p \simeq 147 \) bp. To this end, we combine TPM experiments and dynamical Monte Carlo (DMC) simulations that take into account hydrodynamic effects in the vicinity of the surface. Comparing both data sets, we demonstrate that the surface and the particle do not affect significantly the polymer dynamics provided that the particle radius \( R \) remains small as compared to the DNA contour length. Quantitatively, for the polymers considered in this work, this amounts to \( R < L/6 \). A radius \( R \lesssim 20 \) nm satisfies this condition for all DNA lengths usually studied by TPM. In addition we address rigorously a critical instrumental issue: the detectors used in TPM experiments always have a finite exposure time, ranging from milli-seconds to a fraction of second [10, 11, 18–21]. We show that experimental studies must take into account the finiteness of this exposure time in order to extract valuable relaxation times. Finally, we discuss the intrinsic time resolution of TPM experiments when monitoring DNA conformational changes and give an illustrative numerical example in the case of DNA looping/unlooping.

2. Materials and methods

2.1. Tethered particle motion (TPM) experiments

DNA substrates were obtained by PCR amplification from plasmid templates with a 21-digoxigenin-modified forward primer and a 21-biotin-labeled reversed primer (Eurogentec) as described in [8]. The DNA substrates DNAR401, DNAR798, DNAR1500, DNARL2080 were produced using pAP72 as a template (positions: 1460–1861, 1063–1861, 361–1861, 4625–1861, respectively). Their lengths are \( L = 401, 798, 1500 \) and 2080 bp respectively.

Experiments with neutravidin-coated fluorescent latex particles of radii 20 and 100 nm (Fluospheres, Molecular Probes) were performed using a protocol similar to the one described in [8]. A coverslip flow chamber (30 \( \mu \)L volume) was incubated with the anti-digoxigenin antibody (20 mg L\(^{-1}\); Roche) in phosphate-buffered saline (PBS: 1 mM KH\(_2\)PO\(_4\), 2.97 mM Na\(_2\)HPO\(_4\) and 155.17 mM NaCl) for 20 min at room temperature. After washing, the chamber was incubated with casein (1 g L\(^{-1}\)) in PBS at 4 °C for 4 h. Experiments with the anti-dig-coated fluorescent polystyrene particles of radius 150 nm (anti-dig fluorescent particles, Indicia) were performed using chambers whose surfaces were derivatized with a mixture of polyethylene glycol and biotinylated polyethylene glycol (Sigma, Nanocs) based on the protocol of [22]. The chamber was incubated with neutravidin (20 mg L\(^{-1}\), Molecular Probes) in PBS for 20 min at room temperature. In both cases, the chamber was subsequently rinsed and incubated with the mix of heterobi-functionalized DNA and neutravidin (20 nm, 100 nm) or anti-dig (150 nm)-coated particles (ratio 1:1, 1 pM) that had been prepared 1 h before in PBS with 0.1 g L\(^{-1}\) BSA. Due to the lack of small particles coated with anti-dig, we could not use the PEG-passivated coverslips, known to reduce unspecific binding of particle–DNA complex on the glass, under all the conditions.

The fluorescent particles (radii 20 and 100 nm fluorospheres neutravidin from Molecular Probes and radii 150 nm anti-dig fluorescent particles from Indicia) were characterized by...
dynamic light scattering (Protein Solutions Dynapro MS-800). After sonication, as usually performed before their use in TPM, the different particles were found to exhibit a narrow distribution of hydrodynamic radii $R_H$ (41 ± 1 nm and 120 ± 5 nm for the latex particles of radii 20 nm and 100 nm, 153 ± 4 nm for the polystyrene particles). The observation of aggregated particles can thus be excluded: the similar 20 nm difference measured between the geometrical and the hydrodynamic radii of the 20 and 100 nm particles is probably due to the adsorption of several layers of neutravidin.

The tethered particles of 150 nm (resp. 100 nm and 20 nm) were visualized at room temperature by use of a fluorescence video microscope, equipped with a 63× objective and an additional 4× magnification lens leading to a 252× magnification, on a CCD camera Coolsnap (resp. Cascade, RoperScientific) at a recording time of 25 frames s⁻¹ (resp. 74 frames s⁻¹ and 112 frames s⁻¹), which corresponds to an acquisition period $T_aC$ equal to 40 ms (resp. 13.5 ms and 8.9 ms). In all cases, we could restrict the exposure time $T_{ac}$ ≤ $T_a$ to 5 ms by the use of an AOTF, characterized by an extinction ratio exceeding 7400 (or by entering such a command in the acquisition parameters of the Cascade CCD). Measurements with 2080 bp long DNA molecules and $R = 150$ nm particles were performed on both cameras showing very good agreement on all the analyzed parameters (1.7% difference at most).

The two-dimensional projection $P_{1}$ of the particle position was determined on successive images as the spot centroid using a home-built image analysis program (Labview). The accuracy of the position detection (pointing error), calculated as the standard deviation of the positions of immobilized particles accumulated during 30 s, is equal to 36, 21 and 8 nm for the particles of radius 20, 100 and 150 nm, respectively. Trajectories have an average duration of 90 s. Along a trajectory, the DNA anchoring point at time $t$ is determined by averaging the particle position over an interval of duration $T_{av} = 2$ s centered at $t$, and then subtracted from $P_{1}$. This sets $(P_{1}) = 0$ and subtracts the instrumental drift. Since $T_{av}$ is much larger than the diffusion relaxation time (see below), this anchoring point is determined with a good accuracy. The trajectories exhibiting asymmetry were discarded following [23]. The amplitude of movement of the particle is defined as the variance of $P_{1}$ averaged on the trajectory as

$$\Delta r_{\parallel}^2 = \langle r_{\parallel}^2 \rangle. \quad (1)$$

A minority of trajectories appeared to have an amplitude of movement $\Delta r_{\parallel}$ significantly shorter than the majority ones, which yields a bimodal distribution of $\Delta r_{\parallel}$ with a small population of low amplitudes. These trajectories, that might be related to multi-DNA–particle complexes, have been discarded. The final number of trajectories for each condition ($L, R, T_{ex}, T_{ac}$) ranges from 19 to 60. Details are given in the supplementary data (available at stacks.iop.org/PhysBio/7/046003/mmedia).

2.2. DNA coarse-grained model

The labeled DNA polymer is modeled as a chain of $N$ connected small spheres of radius $a$, whose positions are denoted by $r_i(t)$ where $i = 0, \ldots, N − 1$, and a larger final particle of radius $R ≥ a$, of position $r_f(t)$ (see figures 1 and S2). The DNA contour length is $L = 2a(N − 1)$. In this work, $a$ ranges between 1.4 and 7 nm, and $R$ between 0 (i.e. no particle) and 200 nm. The internal structure of the double-stranded DNA is not considered at this level of modeling. Denaturation bubbles are too scarce at room temperature to have an effect on the global chain conformation [24, 25]. The persistence length value $\xi = 147$ bp is averaged over the nucleotide sequence. Torsional degrees of freedom are omitted as a first hint into the full problem. The polymer is grafted on a surface which sets $r_0 = 0$. We use cylindrical coordinates $(\rho, \varphi, z)$: $r = \rho e_\rho + ze_z = r_1 + ze_z$. Since the polymer motion is limited to the upper half-plane, we impose the following ‘hard wall’ boundary conditions: $z_i > 0$ for monomer spheres and $z > R − a$ for the particle. We treat a freely rotative joint to the glass coverslip [9], by fixing the first sphere center at a height $a$ above the surface (also see the supplementary data available at stacks.iop.org/PhysBio/7/046003/mmedia).

All spheres interact via stretching and bending forces: the potential $U$ is the discrete version of the extensible worm-like chain potential and depends on the sphere positions $r_i$ and the particle one $r$ as $U = \sum_{i=0}^{N-2} \left( |r_i − r_{i+1}| − 2a \right)^2 + \frac{1}{2} \left( r_1 − r \right)^2 + \frac{1}{2} \left( r_R − r \right)^2$. The backbone and $\sum_{i=1}^{N-1} \left( r_1 − r_i − r_{i+1} \right)^2 + \frac{1}{2} \theta_i \left( r_1 − r_i − r_{i+1} \right)^2 + \frac{1}{2} \theta_i \left( r_R − r_i − r_{i+1} \right)^2$, where $\theta_i$ is the angle between neighboring bonds of sphere $i$. The first term ensures the polymer connectivity and the second term is the bending energy with zero spontaneous curvature. The last two terms are dedicated to the particle. The parameters $\gamma$ and $\varepsilon$ are the stretching and bending moduli [26]. The persistence length is given by $\xi_p = \varepsilon/(\k_B T)$. We choose $\gamma a^2 = 4\varepsilon$, which is exact for an isotropic elastic cylinder with radius $a$. Therefore the key parameters in the simulation are $L/\xi_p$, $R$ and $N$. Mutual penetration of monomers is prevented by an excluded volume interaction.

A fully realistic description would be to fix the sphere radius, $a$, equal to the DNA half-width, i.e. about 1 nm. Hence one sphere would model roughly 6 bp, and 25 spheres would correspond to the typical DNA persistence length of 147 bp at physiological temperature and salt concentration. Time limitation in the simulations led us to concentrate on $N = 50$ (or 25) for a DNA of 400 to 2000 bp and thus to choose $a > 1$ nm. As far as equilibrium properties are concerned, the DNA statistical mechanics are insensitive to the choice of $N$, provided that $a$ remains small as compared to $\xi_p$, so that a sphere represents a DNA segment that is actually rigid.

2.3. Dynamical Monte Carlo simulations

Out-of-equilibrium dynamics can be tackled numerically by DMC simulations that become equivalent to Brownian dynamics when the variation of energy at each time step, $\Delta U$, satisfies $\Delta U \ll k_B T$ [27]. We have also performed Brownian dynamics simulations of the system which yield very similar results on equilibrium and dynamical properties (see the supplementary data available at stacks.iop.org/PhysBio/7/046003/mmedia). At each Monte Carlo step (MCStep) of physical duration $\delta t$, a bead is chosen uniformly at random among the $N + 1$ possible ones (monomer
spheres and labeling particle). Then a random move \( \delta r \) is attempted for this bead, uniformly in a ball of radius \( R_0 \), thus \( (\delta r)^2 = 4\pi \int_0^{R_0} r^2 \, dr/4\pi \int_0^{R_0} r^2 \, dr = (3/5)R_0^2 \). This quantity must be equal to \( 6D_0 \delta t \), where \( D_0 \) is the diffusion coefficient of the spherical bead, depending on its diameter.

In practice, for monomer spheres, \( R_0 = a/5 \) (unless stated differently), where \( a \) is their radius. Then \( \delta t \) is set subsequently through \( \delta t = R_0^2/(10D_0) \), and, for the particle, \( R_0' = \sqrt{10D_0 \delta t} \) is fixed with \( D_0 = D_0a/R \). Interactions between adjacent beads are treated via the interaction potential energy \( U \), whereas interactions between non-adjacent beads are of hard core nature, like surface-bead interactions: whenever a move would lead to the penetration of a bead into an other one or the surface, it is rejected. A Monte Carlo sweep (MCS) is a sequence of \( N+1 \) MCS steps. The physical time is incremented of \( \delta t \) following each MCS [27]. In other words, on average, each bead moves once per MCS, i.e. per physical time interval \( \delta t \). Typically, a simulation lasts between \( 10^9 \) and \( 10^{10} \) MCS, so that the simulation time is typically larger than 100 times the relaxation time. This leads to satisfactory error bars, estimated by usual techniques [27] (see also equation (12)). In the simulations, \( N = 50 \) or 25, depending on the slowness of the physical process. Since \( a \ll \ell_s \) in all cases, we expect that dynamics do not depend on \( N \) either. A simulation snapshot is shown in figure 1. Finally, note that in our DMC simulations, moves are local (one bead at once). This choice is more time-consuming than the global Monte Carlo sampling of [10, 28], but has the advantage to give access to dynamical properties, which is the main goal of this work.

2.4. Diffusion near the surface: hydrodynamic effects

The motion of a spherical particle is slowed down near a flat surface due to the no-slip condition for the solvent velocity flow at the wall. The induced hydrodynamic interactions cause a variation, with the distance to the surface, of the diffusion coefficient as compared to its bulk value \( D_0 \). This new diffusion tensor can be split into a component parallel to the wall, \( D_{||} \), and a perpendicular component, \( D_{\perp} \). For a sphere of radius \( b \) (here \( b = a \) or \( R \)), the center of which is at a distance \( h \) from the wall, parallel and perpendicular diffusion coefficients are derived from Faxén’s law [29]. At order 3 in \( b/h \)

\[
D_{\perp} = D_0 \left( 1 - \frac{9b}{8h} + \frac{b^3}{2h^3} \right),
\]

\[
D_{||} = D_0 \left( 1 - \frac{9b}{16h} + \frac{b^3}{2h^3} \right). \tag{2}
\]

Monte Carlo simulations must be modified to take into account these spatially varying diffusion coefficients. First of all, random moves \( \delta r \) are now randomly chosen in an ellipsoid to account for anisotropy. In addition, careful attention must be paid to the discretization of the equations of motion in this case. A vertical drift term \( (dD_{||}/dh) \) \( e \) must be added to compensate the variation of \( D_{||} \) with \( h \) to restore the detailed balance condition [30]. As a consequence, this improvement of dynamics does not affect equilibrium properties such as chain statistics.

2.5. Extracting relaxation times from experimental and numerical data

The relaxation time \( \tau_1 \) associated with \( \tau_i \) can be defined through the two-time correlation function averaged over a trajectory

\[
C(t) = \langle r_1(s+t) \cdot r_1(s) \rangle_s \approx |r_1|^2 \exp(-t/\tau_1), \tag{3}
\]

if one assumes without loss of generality that \( \langle r_1^2 \rangle = 0 \), or through the 2D mean square deviation (MSD):

\[
\text{MSD}(t) = \langle (r_1(s+t) - r_1(s))^2 \rangle_s = 2\langle r_1^2 \rangle - 2C(t) \approx 2\langle r_1^2 \rangle[1 - \exp(-t/\tau_1)]. \tag{4}
\]

At short times \( t \ll \tau_1 \), one expects to recover the 2D diffusion law \( \text{MSD}(t) = 4Dt \), with \( D \) the apparent particle 2D diffusion coefficient. Thus the correlation time for this particle in a 2D trap with variance \( \Delta r^2 \) is taken to be \( \tau_1 = \Delta r^2 / 2D \). \tag{5}

For simulated trajectories, the relaxation times are fitted from \( C(t) \) and \( \text{MSD}(t) \), using equations (3) and (4), leading to two relaxation times, \( \tau_C \) and \( \tau_{\text{MSD}} \). Below, we report the mean values \( \tau_m = (\tau_C + \tau_{\text{MSD}})/2 \), with error bars taken as \( |\tau_C - \tau_{\text{MSD}}|/2 \). In practice, fits are performed on an interval \( t \in [0, t_{\text{trap}}] \). Since numerical error bars on \( C(t) \) and \( \text{MSD}(t) \) are larger and larger as \( t \) grows, the smallest possible value of \( t_{\text{trap}} \) must be used. On the other hand, for \( \text{MSD}(t) \), one must have \( t_{\text{trap}} \) larger than a few \( t_{\text{MSD}} \) in order to fit properly the exponential decay. We have chosen \( t_{\text{trap}} = 4t_{\text{MSD}} \), which is a good compromise between both constraints. The fitting procedure, consisting in fitting \( \tau_{\text{MSD}} \) on \( [0, t_{\text{trap}}] \) and then adjusting \( t_{\text{trap}} = 4t_{\text{MSD}} \), is iterated a few times until \( t_{\text{MSD}} \) is converged. Similarly, \( \tau_C \) is obtained by measuring the slope of \( \ln[C(t)/C(0)] \) \( \approx -t/\tau_C \), on the interval \( [0, \tau_C] \), with the same iterative procedure. However, in some instances \( R \leq 20 \text{ nm} \), \( \ln[C(t)/C(0)] \) appears to display a short transient, equal to a small fraction of \( \tau_C \), because of slow diffusion modes. In this case, linear regressions are performed on a suitably chosen interval \( [t_{\text{trap}}/2, \tau_C] \), where they appear to be very good (correlation coefficients \( |r| > 0.9995 \)).

For experimental data, \( \tau_1 \) is fitted from \( C(t) \) as follows. The raw correlation function averaged over all available trajectories is denoted by \( C_{\text{raw}}(t) \). The systematic pointing error is taken into account: the detected position, \( r_{\text{det,raw}} \), is the sum of the actual position, \( r_1 \), and the pointing error, \( r_e \), two independent random variables. Thus \( \Delta r_{1,\text{raw}}^2 = \langle [r_{1,\text{raw}}]^2 \rangle = \langle r_1^2 \rangle + \langle r_e^2 \rangle \). The second contribution is systematically subtracted from measured values \( \Delta r_{1,\text{raw}}^2 = C_{\text{raw}}(0) \), using the pointing error values as given above. This modified correlation function is denoted by \( C_m(t) \). In addition, the subtraction of drift induces systematic anti-correlations at short times leading to the following fitting form

\[
\frac{C_m(t)}{C_m(0)} = 1 + \frac{\tau_m}{T_w} e^{-t/\tau_m} - 2\frac{\tau_m}{T_w}, \tag{6}
\]

with a single fitting parameter, \( \tau_m \). This fitting form is demonstrated in the supplementary data (equations (S1–3) available at stacks.iop.org/PhysBio/7/046003/mmedia).
Examples are given in figure S1. The prescription is the same as above: the fitting interval $[0, t_{sup}]$ is chosen so that $t_{sup} = 4 r_m$. Error bars on $r_m$ correspond to the standard deviation of the measurements on individual trajectories.

### 2.6. Correction of detector time-averaging effects

Finally, one has to correct time-averaging effects in experimental results. In [10, 11, 18–21], the time-averaging (or blurring) effect due to the finite exposure time of detectors in single molecule (or particle) tracking experiments was investigated. When tracked molecules or particles diffuse in confined regions, diffusion constants can be significantly underestimated, as well as sizes of confining domains. This effect was quantified by exact analytical arguments, in the contexts of diffusion in membrane domains [18] and optical effect was quantified by exact analytical arguments, in the limiting cases, rigid rod ($\rho = 0$) and flexible case ($2 \rho < l_p < 14$) for which no analytical expression for $p(\rho)$ is known. The theoretical study of the limiting cases, rigid rod ($\rho \leq l_p$) and flexible chain ($\rho \geq l_p$), is reported in the supplementary data (available at stacks.iop.org/PhysBio/7/046003/mmedia). In addition to the experimental particle radii $R = 20, 100$ and $150$ nm, we also examined numerically the radii $R = 40, 80$ and $200$ nm, as well as $R = 0$ (no particle). The numerical distributions $p(\rho)$ and their deviation from a Gaussian are discussed in the supplementary data (figures S4–S6, available at stacks.iop.org/PhysBio/7/046003/mmedia).

The two distributions $p(\rho)$ extracted from both TPM experiments and simulations are compared in the supplementary data (see figures S5 and S6, available at stacks.iop.org/PhysBio/7/046003/mmedia). The agreement is systematically good, even though some small discrepancies appear. In order to quantify them, we plot in figure 2 the values of $\Delta r||$ as a function of $L$. Interpolating functions evaluated from Monte Carlo simulations have been proposed in [10] that give $\Delta r||$ in function of $L, R$ and $l_p$. When $R \geq 100$ nm, we have checked that our numerical results are in agreement with these interpolations, even though they were calibrated for $R > 190$ nm. In figure 2, one observes that experimental $\Delta r||$ are shorter than simulated ones. Nelson et al [10] have already noted that assuming the persistence length $l_p = 147$ bp (50 nm) in data analysis leads to larger $\Delta r||$ than observed. To circumvent this issue, they pointed out that setting $l_p = 128$ bp (43.4 nm) yields better agreement, attributing this low value to the buffer. We also observe that setting $l_p = 128$ bp leads to values of $\Delta r||$ closer to our experimental observations for $R = 150$ nm.

However, for $R = 20$ nm, the interpolating functions proposed in [10] are less reliable (see figure 2). In particular, decreasing the persistence length value to $l_p = 128$ bp is no
longer adequate and goes into the wrong direction (147 bp is a better choice).

Note that the actual value of $\ell_p$ is certainly not the unique source of discrepancy between theory and experiments. For example, the interaction between the functionalized surface on the one hand, and the particle (and DNA) on the other hand, is more complex than a simple excluded volume one. Therefore inferring a precise value of $\ell_p$ from such experiments is a challenging task [12, 31]. Given the approximations used in the physical modeling, we conclude that the agreement with experiments (discrepancies are less than 15%) is satisfactory.

3.2. Relaxation times of the particle–DNA complex

In this section, we compare the relaxation times $\tau_i$ extracted from both experimental and numerical trajectories (see section 2). We first present theoretical considerations on the dynamical properties of a DNA fluctuating freely in solution and then give basic insights into the influence of the attached particle. We finally address the issue of the whole particle–DNA-surface system, both experimentally and numerically.

3.2.1. Theoretical aspects on relaxation times and diffusion coefficients. For a free chain of polymerization index $N$ and radius of gyration $R_G$, the correlation time is [32]

$$\tau_{\xi} \simeq \frac{R_G^2}{D_{\text{chain}}} = \frac{NR_G^2}{D_0}. \quad (9)$$

In the second equality, we have used the Rouse model ignoring hydrodynamic interactions and valid for flexible chains ($L/\ell_p \gg 1$), which sets that $D_{\text{chain}} = D_0/N$ where $D_0 = k_BT/(6\pi \eta a)$ is Stokes’ formula for the diffusion coefficient of a monomer sphere of radius $a$ in a liquid of viscosity $\eta$. For a Gaussian chain, $R_G^2 \propto N$ and we get $\tau_{\xi} \sim N^2/D_0$ at large $N$. For a rigid rod, $R_G^2 \propto N^2$ and $\tau_{\xi} \sim N^2/D_0$.

Note that at the level of modeling chosen in the present work where the real polymer is modeled by $N$ connected monomer spheres, dynamics (and thus $\tau_{\xi}$) is not affected by the choice of $N$ as soon as $a \ll \ell_p$. Indeed, $R_G$ being insensitive to the choice of $a$ (or $N$), the only dependence in $N$ comes from the ratio $N/D_0$ in equation (9). But with the definition of $D_0$, $N/D_0 \propto Na \propto L$, independently of $a$.

How is this chain relaxation time modified when a particle is grafted at one end? The time evolution of the DNA polymer and the particle is governed by the over-damped Langevin equation [26, 32]

$$\xi \mathbf{f}_i(t) = -\nabla U(\mathbf{r}_0, \ldots, \mathbf{r}_{N-1}, \mathbf{r}_{\text{part}}) + \xi_i(t), \quad (10)$$

where $\mathbf{r}_i$ are the position of spheres ($i = 0, \ldots, N-1$) and the particle ($i = \text{part}$) (we neglect hydrodynamic interactions as a first hint into the full problem). This equation relates the linear response of each object $i$ to forces applied to it: on the one hand, the derivative of the potential $U$ (sum of pairwise potentials) between the $N$ objects; on the other hand, the stochastic forces $\xi_i(t)$ mimic the action of a thermal heat bath and obey the fluctuation–dissipation relation: $\langle \xi_i(t) \cdot \xi_j(t') \rangle = 6k_BT\xi_i \delta(t-t')\delta_{ij}$, where the friction coefficient $\xi_i = \xi_0$ for monomer spheres and $\xi_0 R/a$ for the particle) is related to the diffusion coefficient $D_i$ through $D_i = k_BT/\xi_i$.

We relate the diffusion coefficient of the particle–DNA complex, $D_c$, to those of the particle alone, $D_{\text{part}}$, and the DNA polymer alone, $D_{\text{chain}}$. To get rid of the interaction forces, we consider the barycenter of the particle–DNA complex $r_c = (\xi_{\text{chain}} \mathbf{r}_{\text{chain}} + \xi_{\text{part}} \mathbf{r}_{\text{part}})/\xi_c$ where $\xi_{\text{chain}} = \sum_i \xi_i/N$ is the DNA center of mass. Then a linear combination of Langevin equations (10) yields $\xi \mathbf{f}_c(t) = \xi_c(t)$, where $\xi_c = \xi_{\text{chain}} + \xi_{\text{part}}$. The correlation function $\langle \xi_c(t) \cdot \xi_c(t') \rangle = 6k_BT\xi_c \delta(t-t')$ sets the diffusion coefficient of the particle–DNA complex, $D_c$:

$$D_c^{-1} = D_{\text{part}}^{-1} + D_{\text{chain}}^{-1}. \quad (11)$$

We emphasize that $D_c$ should not be confused with the measured effective particle diffusion coefficient, $D$, because $r_c \neq r_{\text{part}}$. The only case when they are equal is for $D_{\text{part}} \ll D_{\text{chain}}$. By contrast, the relaxation time $\tau_{\xi}$, obtained by tracking the particle only, is a feature of the dynamics of the whole complex. This is the reason why we focus on this observable in the following.

3.2.2. Simulation results without hydrodynamic interactions. We first display in figure 3 the relaxation times fitted from numerical trajectories simulated without hydrodynamic interactions with the surface. They are denoted by $\tau_{\xi}^{\text{sim}}$. We checked that finite-$N$ effects appear to be negligible for $a \ll \ell_p$ (data not shown). We also explored the reference case $R = 0$, where no particle is attached to DNA. We have seen above that the Rouse model predicts $\tau_{\xi} \sim L^2$ at large $L$. Figure 3 shows that this regime is also a good approximation even for the finite sizes considered here. We also plot in figure 3 the relaxation times in the case where $R = 0$ in the absence of hard wall at $z = 0$. One can see that the wall does not hinder DNA dynamics significantly.

When $R > 0$, the particle slows down dynamics because viscous drag increases with the particle size. However our simulations predict that DNAs are not significantly affected by a small particle ($R = 20$ nm). A medium particle, $R = 40$ nm (resp. 80 nm) does not perturb DNAs of lengths $L \geq 800$ bp (resp. 1500 bp). In the other cases, increasing $R$ from 0 to 150 nm at fixed $L$ monotonically increases $\tau_{\xi}^{\text{sim}}$ by a factor ranging from 2 ($L = 2080$ bp) to 10 ($L = 401$ bp).

In the supplementary data (see figure S8, available at stacks.iop.org/PhysBio/7/046003/mmedia), we compare our numerical values of figure 3 without wall hydrodynamic friction to simple calculations where the DNA molecule is modeled by an ideal spring. In this case, the relaxation time would be equal to the ratio of the particle (or particle–DNA complex) friction coefficient to the DNA spring constant [12]. This approach appears to be insufficient because the interplay between the polymer, the particle and the wall is more complex than this simple image.

3.2.3. Simulation results with hydrodynamic interactions with the surface. Hydrodynamic interactions between the particle–DNA complex and the surface are implemented in the numerical code using equation (2). Figure 3 shows that the diffusion is slowed down by hydrodynamic corrections for...
large particles (R ≥ 80 nm) but is weakly affected for small ones (R ≤ 40 nm). Indeed, small spheres are far away from the surface, in that sense that R ≪ z and b/h = R/(z + a) is generically small in equation (2). Only the first DNA spheres, for which b/h = a/(z + a) is of order 1, are slowed down by the proximity of the surface but this is not sufficient to affect the whole dynamics. This is related to the observation that the exact modeling of the DNA-surface joint is not a relevant issue (see the supplementary data available at stacks.iop.org/PhysBio/7/046003/mmedia). By contrast, when R is large, R/(z + a) is close to 1 for the particle itself, and hydrodynamic corrections play a significant role. In the following, we use these numerical results with hydrodynamic interactions.

3.2.4. Comparison between experimental and numerical relaxation times. Figure 4 displays our experimental (corrected from detector averaging effects using equation (7)) and numerical values of the relaxation times τ∥ extracted from functions C(t) or MSD(t) (see section 2). Experimental and numerical values are found in good agreement, with ratios of experimental to numerical values varying from 0.5 to 2. These ratios are plotted in the inset of figure 4. They appear to be well correlated with the ratios L/R and to follow the approximate power law: τ∥ \text{exp}/τ∥ \text{num} \propto (R/L)^{1/3}. This suggests that the observed discrepancies are not due to statistical errors (as supported by the small error bars), but have a physical origin. When R ≪ L, τ∥ \text{exp} < τ∥ \text{num}, i.e. experiments are faster than simulations. If R ≥ L/5, then τ∥ \text{exp} ≥ τ∥ \text{num}. Indeed, if one neglects hydrodynamics, the measured diffusion coefficient of the particle–DNA complex, D∥, is related to the particle one, D∥, and to the polymer one, Dchain, through equation (11): $D\parallel = D\text{part} + D\text{chain}$, and $D\parallel^{-1} = D\text{part}^{-1} + D\text{chain}^{-1}$. This suggests that the particle does not slow down the complex provided that $D\text{part} \gg D\text{chain}$. Now $D\text{part} = K/R$ with $K = k_BT/(6\pi \eta)$, and $D\text{chain} = K/(N\alpha) = 2K/L$ in the Rouse approximation. If 2R ≪ L, then $D\text{part} \gg D\text{chain}$ and the DNA dominates the particle–DNA dynamics, as if the particle were absent. But in this case, hydrodynamic interactions between DNA segments should be taken into account. Since such interactions are known to accelerate polymer dynamics [26, 32], one expects $\tau_\parallel$ to be shorter than in simulations, as indeed observed in experiments. Conversely, if 2R ≫ L, then the particle dominates the particle–DNA dynamics, as if the role of the DNA polymer was limited to its spring properties. We have simplified the complex hydrodynamics of this particle in the vicinity of a wall through the expansions in equation (2). But these expansions are valid in the small b/h limit only and we cannot expect them to be correct when b/h ∼= 1, which is the case when 2R ≫ L. In [29] section 7-4, it is proved that equation (2) overestimates $D_∥$ and $D_\parallel$, which is the case for $2R \ll L$. Thus $\tau_\parallel$ is underestimated as observed. Describing correctly the particle dynamics would require a full integration of all hydrodynamic interactions, between the monomer spheres, the particle and the surface. This is beyond the scope of this work.

In experiments, particles are coated by proteins, which is likely to increase their radius R. This point deserves to be discussed. This increase of R can have two main consequences, both leading to an increase of $\tau_\parallel$: (i) the distance between the last DNA sphere and the particle center can be larger than expected; (ii) the drag force on the particle can also be larger than expected. In particular, the hydrodynamic radius $R_H$ of the 20 and 100 nm particles (coated by...
neutravidin) measured in a solution by dynamic light scattering is 20 nm larger than the bare particle radius (see section 2).

For the $R = 150$ nm particles coated by anti-dig antibodies, this point is less crucial because the anti-dig layer is much thinner. But taking into account rigorously these two effects in simulations are delicate. Point (i) depends on the exact way DNA is attached to proteins, which is unknown. Point (ii) could be solved by replacing $R$ by $R_H$ when calculating the diffusion coefficient of the particle, even though this is a crude approximation. Indeed the protein-coated particle cannot be seen as a solid sphere no longer, which changes the boundary conditions for the velocity field at the decorated particle surface, in particular when calculating $D_{L}$ and $D_{⊥}$. To quantify the effects of protein coating, an upper bound of its consequences is given by simulations where $R$ is replaced by $R_H$. The effect on $\Delta r_{L}$ is a weak increase (10% in the worst case $R = 20$ nm and $L = 401$ bp, 4% in the most favorable case $R = 100$ nm and $L = 2080$ bp). Concerning $t_{1}$, for $R = 20$ nm particles, simulations with $R = 40$ nm (figure 3) show that its increase varies from 40% for $L = 798$ bp down to 16% for $L = 2080$. Interpolating between $R = 100$ and 150 nm provides a good estimate of $t_{1}$ for $R = 120$ nm. The increase goes from 36% down to 10%. This upper bound does not modify our conclusions, even though the disagreement between experiments and simulations for 20 nm particles is slightly worse. By contrast, the fit with a $-1/3$ exponent in the inset of figure 4 is improved (data not shown).

3.3. Choice of TPM parameters to infer DNA dynamics

The preceding analysis leads us to suggest the following recommendations to extract the most valuable information on DNA dynamics from TPM experiments.

3.3.1. Small particles of radius $R \lesssim 20$ nm do not slow down DNAs as short as 400 bp. Comparing simulation data for $R = 0$ and $R > 0$ (figure 3) shows that the presence of the particle does not increase the relaxation times for small particles or long DNAs. Indeed, provided that $R \ll L/2$, dynamics are dominated by the polymer. Accordingly, figure 3 shows that if $R < L/6$ then $t_{1}$ is less than twice the relaxation time of DNA without particle, which demonstrates that slowing down is weak or moderate in this case. In particular, slowing down is very weak when $R = 20$ nm for $L = 401$ to 2080 bp.

They are three contributions to hydrodynamics: (i) slowing down of the chain and the particle, due to the no-slip condition at the surface, (ii) hydrodynamic interactions between the chain and the particle and (iii) interactions between monomer spheres. For creeping flows, these three contributions are additive [29]. We have shown above that, for small $R$, (i) affects only very weakly the relaxation times. Contributions (ii) and (iii), which are not taken into account in simulations, moderately accelerate the dynamics of the particle–DNA complex, as corroborated by experimental measurements (diamonds in figure 4). By linearity, we thus expect that neither the wall nor the small particle slows down DNA dynamics as compared to a DNA chain fluctuating freely in the solution.

### Table 1. Relaxation times and movement amplitudes before ($t_{m}$ and $\Delta r_{L,m}$) and after correction ($t_{1}$ and $\Delta r_{L}$) of detector time-averaging effects, using equations (7) and (8), for two DNA lengths $L$ and two exposure times $T_{ac}$.

| L (bp) | $T_{ac}$ | $T_{ex}$ | $t_{m}$ | $\Delta r_{L,m}$ | $t_{1}$ | $\Delta r_{L}$ |
|-------|---------|---------|--------|----------------|--------|----------------|
| 2080  | 40      | 40      | 69.5   | 223            | 56.1   | 250            |
| 400   | 40      | 5       | 59.1   | 258            | 57.4   | 261            |
| 12.8  | 5       | 60.1    | 261    | 58.4           | 264    |
| 401   | 40      | 40      | 32.8   | 91             | 19.5   | 122            |
| 40    | 5       | 21.7    | 118    | 20.0           | 123    |

3.3.2. Taking into account exposure times and acquisition periods. In section 2, we have explained how the finiteness of the exposure time, $T_{ex}$, modifies the root-mean-square excursion of the particle, $\Delta r_{L}$, the particle–DNA relaxation time, $t_{1}$, and the effective particle diffusion coefficient, $D$, (when $T_{ac} \leq 3t_{1}$). We have prescribed how to recover their real values rigorously, contrary to the rough approximation of equation (8) proposed by [111]. Indeed, the formula given in this anterior work largely overestimates the averaging effect, thus requiring the introduction of a phenomenological time scale $\tau^{*}$.

To this respect, we have analyzed two particle–DNA complexes ($R = 150$ nm and $L = 2080$ and 401 bp) with two different exposure times, $T_{ex} = 5$ and 40 ms and the same acquisition period $T_{ac} = 40$ ms in both cases (and in addition $T_{ex} = 5$ ms and $T_{ac} = 12.8$ ms for $L = 2080$ bp). The measured values before and after applying equations (7) and (8) are given in table 1. We observe that the corrected values are in excellent agreement for both DNA lengths. These values validate our method to correct detector averaging effects of the experimental data.

The values of $t_{1}$ given in figure 4 will help experimentalists to anticipate the appropriate required values of the exposure time $T_{ex}$ in future works. Owing to the complexity of the problem, no simple formula can be given that expresses $t_{1}$ as a function of $R$ and $L$. In practice, a linear interpolation of the values of figure 4 will provide a good estimate of $t_{1}$ for other values of $R$ and $L$, in the range studied.

However, reducing $T_{ex}$ may remain insufficient when one is interested in measuring $t_{1}$. The acquisition period $T_{ac}$ being imposed by the camera, the only points available to fit $MSD(t) \propto 1 - \exp(-t/t_{1})$ or $C(t) \propto \exp(-t/t_{1})$ plots are measured at discrete values $t = 0, T_{ac}, 2T_{ac}$ and so forth. So if $T_{ac}$ is large as compared to $t_{1}$, $MSD(T_{ac})$ and $C(T_{ac})$ have already reached their asymptotic values, respectively $2\Delta r_{L}^{2}$ and 0, making any fitting procedure hopeless. If one is interested in dynamical properties, the true technical limit obviously remains the camera acquisition period, which must be of the same order of magnitude as $t_{m}$ or smaller, typically $T_{ac} \lesssim 2t_{m}$ (as detailed in the supplementary data available at stacks.iop.org/PhysBio/7/046003/mmedia).

3.3.3. Measuring variations of $\langle r_{L}^{2} \rangle$ in real time. Beyond the measurement of equilibrium statistics or relaxation rates, TPM experiments can also be used to monitor DNA conformational
changes. To what extent can those conformational changes be monitored in real time? In TPM, such a change is characterized by a variation of the movement amplitude, \( \Delta r^2 \equiv \langle r^2 \rangle \), due to a given molecular event such as protein binding or DNA looping, leading to a variation of the apparent DNA length. But measuring the mean value \( \langle r^2 \rangle \) supposes to average \( r^2 \) on a sufficiently long time interval, of duration again denoted by \( T_w \) [4]. By definition of the relaxation time \( \tau_1 \), the average will be accurate provided that \( T_w \gg \tau_1 \). Once \( \tau_1 \) is known, what averaging time \( T_w \) should be chosen in practice? In other words, what is the minimum time scale of conformational changes that TPM can reach? To simplify the discussion, we assume that the detector-time-averaging effects are weak, i.e. typically \( \tau_1 > T_w \) (see equation (8)). If not, then in this section and the following one, \( \tau_1 \) must simply be replaced by \( \tau_m \) and \( \Delta r^2 = \langle r^2 \rangle \) by \( \Delta r^2_{\tau_m} \).

Formally speaking, \( \langle r^2 \rangle \sim \int_{0}^{\infty} r^2(t) \, dt / T_w \). The relaxation time of \( r^2_{\tau_1} \), \( \tau_{\tau_1} \), is again defined by \( \langle r^2(t) + r^2(t) \rangle - \langle r^2 \rangle^2 \approx \sigma^2 \exp(-t/\tau_{\tau_1}) \) (we use the notation \( \sigma_{\tau_1} \) for the r.m.s. of \( r^2 \), to avoid confusion with \( \Delta r^2 \), the variance of \( r^1 \)). Then the statistical error on the measurement of \( \langle r^2 \rangle \) (68% confidence interval), denoted by \( \sigma_{\langle r^2 \rangle} \), can be quantified as follows [27]: if \( \tau_{\tau_1} > T_w \), then \( \sigma_{\langle r^2 \rangle} = \sqrt{2\tau_{\tau_1}/T_w} \sigma_{\tau_1} \).

This relation takes into account statistical correlations between successive frames, measured by \( \tau_{\tau_1} \). If \( \tau_{\tau_1} < T_w \), then \( \sigma_{\langle r^2 \rangle} = \sqrt{T_w/T_w} \sigma_{\tau_1} \), because all frames are statistically independent.

Now, what is the relationship between \( \tau_{\tau_1} \) and \( \tau_1 \)? Since \( r^1 = x^2 + y^2 \), \( \tau_{\tau_1} = \tau_2 = \tau_2, \) the (identical) relaxation times of the observables \( x^2 \) and \( y^2 \). If \( x \) and \( y \) were Gaussian random variables, then \( \tau_{\tau_2} = \tau_2/2 = \tau_2/2 \). But \( x \) is not exactly Gaussian. In practice, we have measured numerically in our case that \( \tau_{\tau_1} > \tau_1/c \) with \( c \) ranging from 2.5 to 3.5, depending on \( L \) and \( R \). Thus \( \tau_{\tau_1} < \tau_1/2 \).

Furthermore, we have also measured numerically that \( \sigma_{\tau_1} < \langle r^2 \rangle \) for the values of \( L \) and \( R \) studied here. Were \( r^1 \) be exactly Gaussian, a simple calculation shows that \( \sigma_{\tau_1} = \langle r^2 \rangle \) (because \( \tau_1 \) is two dimensional). Thus in the regime where \( \tau_{\tau_1} > T_w \),

\[
\lambda \equiv \frac{\sigma_{\langle r^2 \rangle}}{\sqrt{2\tau_{\tau_1}/T_w}} \leq \sqrt{\frac{T_w}{\tau_{\tau_1}}}. \tag{12}
\]

The equality defines the limiting value \( T_w^\ast = \tau_1/\lambda^2 \). For example, if \( T_w = 10 \) ms and \( \tau_1 \approx 30 \) ms, \( T_w^\ast > 100 \) \( \tau_1 \approx 3 \) s yields a relative statistical error \( \lambda \lesssim 10\% \). If the conformational changes lead to variations of \( \langle r^2 \rangle \) larger than 10% (the statistical noise), then a thresholding method [4, 5, 14, 16] can detect them in real time (provided that their time scale is higher than 3 s). Using shorter DNAs, smaller particles and a faster acquisition device improves the time resolution. Concrete illustrative examples are given in figures S9, S10 and thereafter.

3.3.4. Numerical example of conformational changes detection: DNA looping/unlooping. To further illustrate TPM capabilities in terms of detection of conformational changes, we propose the following example. It is related to TPM experiments where DNA loops are promoted by proteins binding to two specific DNA loci, and DNA conformation alternates between looped and unlooped states [4, 7, 14]. In the looped state, the effective DNA contour length is shorter, resulting in a smaller \( \Delta r^2 \). Switching between looped and unlooped states can be detected in real time provided that their lifetime is long enough. One of the purposes of the present example is to show how the minimum detectable lifetime can be determined a priori from the knowledge of \( \tau_1 \).

We focus on \( L = 798 \) bp and \( R = 20 \) nm with \( N = 25 \) beads (denoted by \( B_0, \ldots, B_{24} \)). Hydrodynamic interactions with the wall are taken into account. The numerical relaxation time is \( \tau_1 = 2.98 \approx 3 \) ms (see figure 3), and \( \Delta r_1 = 124 \) nm. The two loci where protein binding occurs are situated on beads \( B_0 \) and \( B_{18} \). Thus the looped DNA is roughly equivalent to a 400 bp long DNA, with \( \Delta r_1 = 81 \) nm. In the simulation, looping is promoted by an attractive quadratic potential between beads \( B_6 \) and \( B_{18} \): \( V = 2k_B T (r_{18} - r_6)^2 \). When we turn on (resp. off) the potential, the DNA molecule switches into the looped (unlooped) state. In figure 5, \( \Delta r_1^2 \) is plotted in the function of time for different averaging-interval lengths \( T_w \). To set the threshold separating looped and unlooped states, we choose the condition \((1+\lambda)\Delta r_{1,\text{looped}}^2 = (1-\lambda)\Delta r_{1,\text{unlooped}}^2\), leading to \( \lambda = 0.4 \). This means that a maximum relative error of 40% is allowed if one wants to detect unambiguously looped and unlooped states. Using equation (12), this leads to the limiting value \( T_w^\ast = \tau_1 / \lambda^2 = 19 \) ms (provided that \( T_w \leq \tau_{\tau_1} \sim 1 \) ms). As illustrated in figure 5, choosing \( T_w < T_w^\ast \) leads us to numerous false detections of looping/unlooping. In contrast, increasing \( T_w \) above the threshold \( T_w^\ast \) makes false detections unlikely. However, as is shown in figure 5, increasing \( T_w \) increases the error on the determination of the moment when the transition between looped and unlooped states occurs. Hence the looped/unlooped state duration measure is less precise (see the zoom). This precision is on the order of magnitude of \( T_w \). Furthermore, because of averaging, only durations larger than \( T_w \) can be accurately detected. For instance, in figure 5 (zoom), the looped state between \( t = 5.72 \) s and \( t = 5.86 \) s is not detected for \( T_w = 300 \) ms because its duration is shorter than \( T_w \). Therefore \( T_w \) must be large enough to distinguish between looped and unlooped states, but it must be as short as possible to detect the shortest lifetimes [6]. Equation (12) provides the optimal choice. Of course, the shortest \( \tau_1 \), and therefore particles of radius \( R < L/6 \), will provide the best detection capabilities. Another illustrative example is given in figure S8 in the case of DNA bending induced, for instance, by protein binding.

4. Conclusion and outlook

This work focuses on the influence on DNA dynamics of the TPM setup geometry, namely the attachment of the DNA tether ends to the surface and the labeling particle. To what extent DNA characteristic times can be inferred from TPM observations? The answer to this complex issue cannot be summarized in simple formulas because many parameters are involved and interrelated, in particular: the particle size \( R \).
view on DNA–protein interaction processes involving DNA bending [4, 16], looping [4, 7, 14] or migration of a Holliday junction [3] in the future. We have shown in the simulation of looping/unlooping events that the knowledge of the relaxation time $\tau_{\|}$ is a prerequisite in order to extract the most relevant information from TPM experiments exploring DNA conformational changes: if $\tau_{\|}$ is not known, it is not possible to decide whether a detected variation of $\Delta r^2_{\|}$ is a real conformational change or a spurious statistical fluctuation.

On the theoretical side, the scaling law $\tau_{\|}^{\text{exp}} \propto (R/L)^{1/3} \tau_{\|}^{\text{sim}}$ in figure 4 provides a clue that hydrodynamic interactions play a crucial role. Brownian dynamics simulations (see the supplementary data available at stacks.iop.org/PhysBio/7/046003/mmedia) will be the next step in order to elucidate this issue, at least for the fastest complexes (short chains and small beads). They ought to include hydrodynamic interactions between beads [26, 33], usually taken into account through the Rotne–Prager tensor, and hydrodynamic images induced by the presence of the particle and the surface to ensure the no-slip condition on these surfaces [26].

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