Torsional Stiffness of Single Superparamagnetic Microspheres in an External Magnetic Field

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We used magnetic tweezers to measure the torsional stiffness of single micrometer-sized superparamagnetic spheres as a function of the applied magnetic field. By investigating the axial fluctuations of DNA-bound microspheres, we found that considerable rotational microsphere fluctuations can occur. Quantitative noise analysis allowed us to determine the rotational stiffness of individual microspheres, which was found to saturate at high magnetic fields. The saturation can be qualitatively explained considering the properties of the magnetic nanoparticles within the microsphere. Consequences for spatial resolution limits in single-molecule magnetic tweezer experiments and usage of DNA mechanics as a sensitive probe in magnetometry are discussed.

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Micrometer-sized superparamagnetic spheres have become a valuable tool in order to apply force in fundamental biophysics experiments including studies of cellular adhesion [1], ligand-receptor interactions [2] and properties of viscoelastic materials [3]. Most prominently, within so-called magnetic tweezers, superparamagnetic microspheres have been applied to manipulate single biological molecules, such as DNA, and characterize their mechanical properties [4–6]. These investigations set the basis to observe in real time the action of molecular motors that interact with DNA. This provided fascinating insights into the function of such enzyme systems [7,8]. Intriguingly magnetic microspheres exhibit a significant magnetic polarization anisotropy. This can, in addition to linear forces, be used to apply torque and has been exploited to study, e.g., the adenosintriphosphate synthesis by F1-ATPase [9], the twist mechanics [5,6], and dynamics [10] of DNA but also enzymes, which change the DNA twist [7,11,12].

Despite the wide application of torsion and twist generation using magnetic microspheres, the torsional forces and their origin remain inadequately characterized. Depending on the application, torsional forces are either neglected [2] or assumed to be larger than any other acting force [13]. The aim of this study is to provide a quantitative basis and a qualitative understanding for the torsional forces of superparamagnetic microspheres within the applied field. We find that the resolution of magnetic tweezers can become limited by rotational fluctuations of the microspheres. By solving the set of differential equations describing the coupled linear and rotational microsphere motions, we are able to directly quantify the torsional stiffness from the measured fluctuations.

Our experimental setup [13] consisted of a 5.7 kbp dsDNA molecule tethered between a glass surface and a 2.8 μm superparamagnetic microsphere (Invitrogen) widely used for generating high magnetic forces >10 pN [5,6]. A field gradient perpendicular to the surface is generated by permanent magnets. This causes a linear force acting on the microsphere along the gradient direction, while forcing the particle to align in its preferred direction parallel to the surface along the applied field [Fig. 1(a)]. Translating and rotating the magnets allows varying the magnetic force F_{mag} and rotating the microsphere in the plane parallel to the surface. Using a CCD camera (Pulnix 6710-CL) the DNA length is monitored by measuring the z position of the microsphere with respect to a reference sphere at the surface [13].

A typical DNA stretching experiment is shown in Fig. 1(b). The larger the applied force the smaller are the measured DNA length fluctuations, which are always significantly above the detection noise (<0.3 nm root-mean-square amplitude at 60 Hz acquisition [14]).

An analytic expression for the expected noise can be derived by assuming overdamping of the system and approximating the DNA, in the limit of small displacements, as a linear spring with spring constant $k_{\mathrm{DNA}} = dF_{\mathrm{DNA}}/dz_{\mathrm{DNA}}$, which is given by the extensible worm-like-chain (WLC) model [14–16]. The measured dynamics of this simple system of a linear spring and a viscous damping element with a translational drag coefficient perpendicular to the surface γ_{trans} [17,18] is described by the

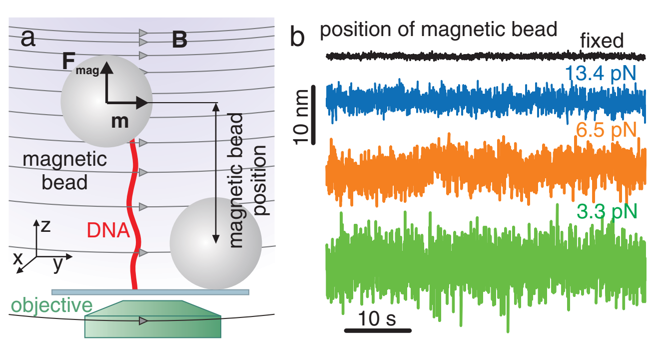

FIG. 1 (color). (a) Schematics of the experimental setup. (b) Time traces of the z position of the magnetic microsphere at different forces. The black trace is for a fixed microsphere.

following noise power spectrum $S_z(f)$ [19]:

$$S_z(f) = \frac{4k_B T\gamma_{\rm trans}}{k_{\rm DNA}^2}\frac{1}{1+(f/f_c)^2}\frac{\sin^2(\pi f/f_{\rm cam})}{(\pi f/f_{\rm cam})^2}, \qquad (1)$$

where k_B denotes the Boltzmann constant, T the temperature, and $f_c = k_{\rm DNA}/2\pi\gamma_{\rm trans}$ the cutoff frequency. The left and middle part of Eq. (1) describe the real occurring fluctuations, whereas the right part corrects for low-pass filtering by the noninterlaced camera with acquisition frequency $f_{\rm cam}$ [19]. Integrating $S_z(f)$ from $0 \rightarrow \infty$ provides the expected mean-square fluctuations $\langle z^2\rangle$ [20].

Comparing the measured with the expected $\sqrt{\langle z^2\rangle}$, we find an excellent agreement at forces <2 pN [Fig. 2(a)], whereas at larger forces a significantly higher noise is measured. However, the extent of this deviation is strongly dependent on the microsphere [compare Figs. 2(a) and 2(b)]. One property, which largely varies between the microspheres, is the off-center attachment $R_\perp$ of the DNA molecule, which originates from the alignment of the magnetic microsphere with the external field. $R_\perp$ can be measured by slowly rotating the magnets and following the induced microsphere rotation in the xy plane, since the microsphere rotates around its DNA attachment point [Figs. 2(c) and 2(d)]. Interestingly $R_\perp$ decreases with increasing force [Figs. 2(c) and 2(d)]. This can be explained by rotational displacements of the microsphere out of its preferred orientation. Such a rotational displacement should, for large $R_\perp$, also be observed along z providing a wrong DNA extension. Indeed force-extension curves for microspheres with large $R_\perp$ did not show the expected WLC behavior [Fig. 2(e)]. Accounting for rotational displacements in z, calculated from the measured $R_\perp$ and the microsphere radius, remarkably restored the force distance curves [Fig. 2(e)]. These rotational displacements suggest a relatively low torsional stiffness $k_{\rm torque}$ of the microspheres within the magnetic field and might explain the observed increase in noise at large forces.

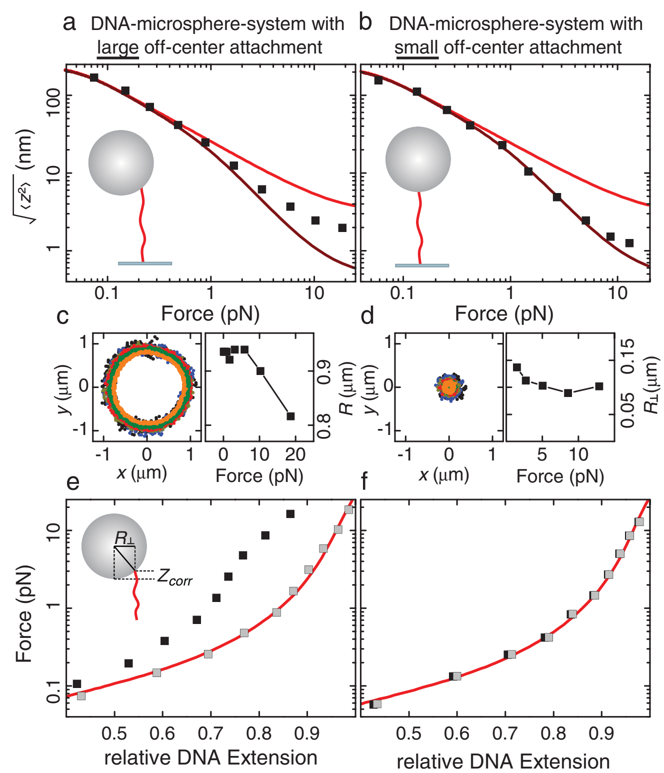

FIG. 2 (color). Noise along z and force-extension relations for a microsphere with large (left) and small (right) off-center attachment $R_\perp$. (a, b) Measured $\sqrt{\langle z^2\rangle}$ as a function of force (black squares). Solid lines are the calculated $\sqrt{\langle z^2\rangle}$ with (upper line) and without correction (lower line) for low-pass filtering by the camera [Eq. (1)]. (c, d) $R_\perp$ measured at different forces by slowly rotating the magnetic field and following the circular track of the microsphere in the xy plane. (e, f) Force-extension curves measured (black squares) and after correction by $Z_{\rm corr}$ (see inset) due to the off-center attachment (gray squares). The solid line is a fit with the extensible WLC model with a persistence length of 45 ± 5 nm.

In order to support this hypothesis, we characterized 27 microspheres with varying $R_\perp$. Indeed, we observe a strong correlation of the observed noise at high stretching forces with $R_\perp$ [Fig. 3(a)]. Evaluating the z deviations due to rotational displacements [see Fig. 2(e)] also provides a strong correlation with $R_\perp$ [Fig. 3(b)]. Taken together, these results indicate that the magnetic microspheres can exhibit significant rotational displacements and fluctuations.

Quantifying the measured fluctuations should allow to determine the torsional stiffness of a single microsphere. To obtain a relation between the torsional stiffness of a magnetic microsphere and the measured $\sqrt{\langle z^2\rangle}$, we derive the power spectrum for the system, in which translational and rotational fluctuations are coupled [Fig. 4(a)] [14]. We approximate the magnetic potential, counteracting the rotational displacements, as harmonic with torsional stiffness $k_{\rm torque}$. The back driving torque is then $T_{\rm mag} = -k_{\rm torque}\Delta\varphi$

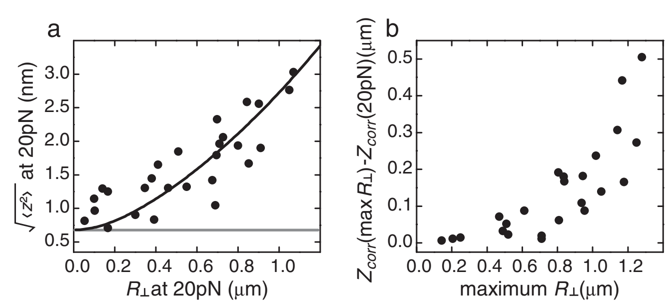

FIG. 3. (a) Measured $\sqrt{\langle z^2\rangle}$ at 20 pN over $R_\perp$. Solid lines represent the expected noise in the absence (gray) and presence (black) of rotational fluctuations of the microspheres for a $k_{\rm torque}$ of 100 pN μm rad^{-1}. (b) z deviations due to rotational displacements [see Fig. 2(e) inset] at 20 pN calculated from the difference in $Z_{\rm corr}$ at 20 pN and at low force where $R_\perp$ maximizes.

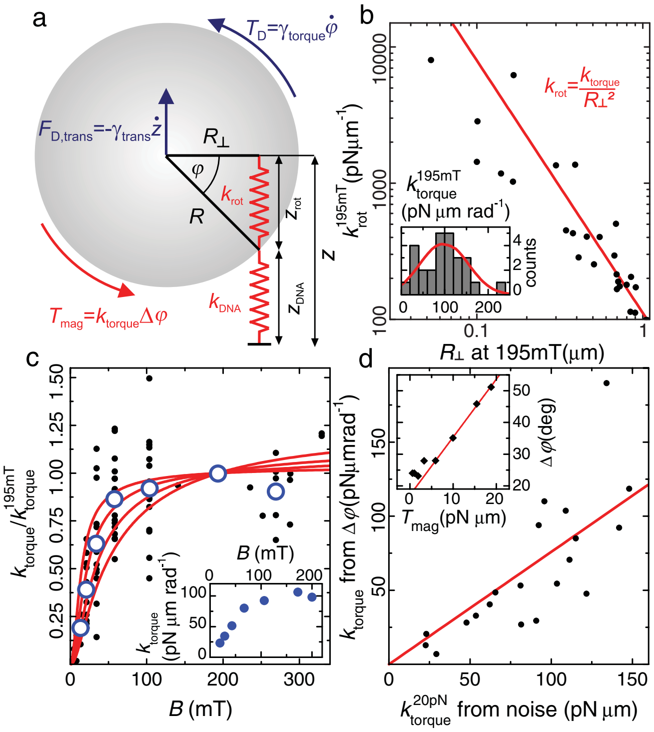

FIG. 4 (color). (a) Sketch of linear, torsional, and drag forces acting on the magnetic microsphere. (b) k_{rot} at 195 mT as a function of $R_\perp$. The red line is a fit to the data with $k_{rot} = k_{torque}/R^n$ with $k_{torque}^{195\,mT} = 111 \pm 4$ pN μm rad^{-1} and $n = 1.9 \pm 0.2$. Inset: histogram of $k_{torque}^{195\,mT}$ with a mean of 94 ± 10 pN μm rad^{-1}. (c) Overlay: all k_{torque} curves as a function of B normalized at 195 mT (black dots). Open circles represent the mean for a given B. Solid lines: calculations for C values of 1, 2, 4, and 7 kJ m^{-3} [14]. Inset: k_{torque} curve of an individual microsphere. (d) k_{torque} estimated from angular displacements (see inset) over k_{torque} from noise measurements. Inset: angular displacement as function of the applied torque for a single microsphere. k_{torque} is estimated from a linear fit to the data at higher forces (solid line).

for small displacements $\Delta\varphi$. The rotational drag torque is given by $T_D = -\gamma_{torque}\dot{\varphi}$ with the rotational drag coefficient γ_{torque} [17]. T_{mag} and T_D originate from corresponding linear forces $F_{torque} = -k_{torque}/R_\perp^2 \Delta z_{rot}$ and $F_{D,torque} = -\gamma_{torque}/R_\perp^2 \dot{z}_{rot}$ along the DNA axis [Fig. 4(a)]. z_{rot} denotes the height of the microsphere center above the DNA attachment point [Fig. 4(a)] with $\Delta z_{rot} = R_\perp \Delta\varphi$ for small $\Delta\varphi$. With the acting random forces $F_{trans}(t)$ and $F_{rot}(t)$, one can now describe the system by the following set of Langevin equations [14]:

$$\begin{aligned} -\gamma_{rot}\dot{z}_{rot} - k_{rot}\Delta z_{rot} + k_{DNA}z_{DNA} &= F_{rot}(t) \\ -\gamma_{trans}\dot{z} - k_{DNA}z_{DNA} &= F_{trans}(t) \end{aligned} \tag{2}$$

with $k_{rot} = k_{torque}/R_\perp^2$ and $\gamma_{rot} = \gamma_{torque}/R_\perp^2$ and $z_{DNA} = z - z_{rot}$. For such a set of coupled linear Langevin equations, one can derive an analytic expression for the noise power spectrum $S_z^{coupl}(f)$ [14,21] and obtain $\langle z^2\rangle_{coupl}$ as described above.

Using the derived expression for $\langle z^2\rangle_{coupl}$, we determined k_{rot} for each microsphere as a function of force. This was done by iteratively finding the k_{rot}, which would generate the observed noise at a given force. For a given force, k_{rot} is decreasing with increasing $R_\perp$ following within error the expected $R_\perp^{-2}$ dependence [Fig. 4(b)]. We also determined the torsional spring constant k_{torque} and its dependence on the magnetic field B [Fig. 4(c) (inset)] measured with a small hall probe [14]. At a given magnetic field, k_{torque} exhibits a significant variability between individual microspheres [Fig. 4(b) (inset)]. To remove scatter, we overlaid the k_{torque} curves from all microspheres measured by normalizing them at 195 mT. Whereas k_{torque} initially increases monotonically with force, it surprisingly saturates at higher forces, suggesting that it becomes independent of the applied field [Fig. 4(c)]. The mean k_{torque} at 195 mT, at which k_{torque} already saturates, equals to 94 ± 10 pN μm rad^{-1} [Fig. 4(b) (inset)]. Calculating the expected noise as the function of $R_\perp$ for the obtained mean k_{torque}, well describes the experimental noise [Fig. 3(a)].

A saturation of k_{torque} is also observed when examining the microsphere angular displacements $\Delta\varphi$ since they arise only when k_{torque} saturates [14]. To quantitatively correlate angular displacements $\Delta\varphi$ with the obtained k_{torque} values, we estimated the saturation value of k_{torque} from plotting $\Delta\varphi$ over the applied torque T_{mag} [Fig. 4(d) (inset)]. The slope of the progressively increasing part of this data at high fields provides then an estimate for the saturating k_{torque}. We find a good correlation between the k_{torque} values determined by the two independent ways [Fig. 4(d)].

The torque experienced by a magnetic dipole within the field is given by $\mathbf{T}_{mag} = \mathbf{mB}$, where $\mathbf{m}$ denotes the magnetic moment. In contrast to our observations, one would therefore expect a linear increase of k_{torque} with B if the magnetic moment has saturated ($B > 100$ mT [14]). This assumes that the magnetization direction is perfectly aligned with the dipole, i.e., anisotropy, axis. Superparamagnetic microspheres consist however of many iron oxide nanoparticles, dispersed in a polymer matrix. Each nanoparticle displays a certain magnetization anisotropy [22]. The magnetization anisotropy of the whole microsphere likely arises from a slight preferential angular orientation of the nanoparticles. Within a single particle the magnetization vector $\mathbf{m}$ can, however, be misaligned from the particle anisotropy axis. For this, an energetic penalty, given by the anisotropy constant C, has to be paid and the free energy density for the particle magnetization within a homogeneous field B can be written as [23]:

$$U_{mag} = \tfrac{1}{2}C\sin^2\alpha - BM\cos(\varphi - \alpha), \tag{3}$$

where M denotes the volume magnetization and φ and α the angles between the anisotropy axis and $\mathbf{B}$ and $\mathbf{m}$, respectively [14]. Equation (3) can be used to calculate k_{torque} assuming a parallel alignment of all nanoparticles of a microsphere [14]. Whereas for small fields **m** is aligned with the anisotropy axis, high fields force an alignment of **m** with the field causing k_{torque} to saturate. A good qualitative agreement with the experimentally observed behavior is obtained for values of C between 1 and 7 $\mathrm{kJ\,m^{-3}}$ [Fig. 4(c)] in agreement with the bulk crystalline anisotropy constant of γFe_2O_3 of 4.7 $\mathrm{kJ\,m^{-3}}$ [22]. Thus, the material properties of the magnetic particles can provide an explanation for the saturation of k_{torque}. However, other effects, such as dipole interactions between nanoparticles, may also contribute.

We now discuss the consequences of our measurements for the application of superparamagnetic microspheres. Because of the rather simple position detection by a camera, the spatial resolution limits of magnetic tweezers are generally believed to be several nanometers [13]. Here, however, we demonstrate that the magnetic microspheres rather than the position detection can be the limiting factor, because of rotational fluctuations. Whereas, for optical tweezers significant resolution improvements have been achieved and discussed [21], hardly any reports about this topic can be found for magnetic tweezers. We hope that this contribution will stimulate a discussion about resolution limits of magnetic tweezers. The simplest way to overcome the influence of rotational fluctuations is the careful selection of DNA-bound microspheres based on their $R_\perp$. However, the development of improved microspheres with either a negligible or a largely increased anisotropy would be a much better alternative.

The obtained values for k_{torque} of $\sim$100 $\mathrm{pN\,\mu m\,rad^{-1}}$ reveal that shear forces induced by torsion can become significantly larger than the obtained stretching forces. k_{torque} is saturated at 195 mT corresponding to $\sim$5 pN stretching force. Depending on the rotational displacement, shear forces of 10 s of pN can easily be caused at the microsphere surface. This can have a tremendous influence in measurements in which the microsphere is rigidly attached to the investigated object, e.g., in studies of cell mechanics [1] and ligand-receptor interactions [2], which need appropriately to be considered.

In summary, we have shown that magnetic microspheres display significant rotational fluctuations within an applied field. These fluctuations can be used to calculate their torsional stiffness as a function of the field. Generally, magnetic measurements of single small particles still remain a challenging topic [24]. With our measurements, we provide a basis for using DNA to carry out magnetic measurements on such systems. Torsion magnetometry is a powerful technique in order to characterize tiny magnetic samples down to torque values of $\sim 10^{-13}$ N m [25]. We reach here a sensitivity of $\sim 10^{-17}$ N m. However, even more sensitive measurements can potentially be achieved by using the torsional spring constant of DNA directly. We therefore speculate that DNA-based magnetic tweezers might become a new tool in ultrasensitive torsion magnetometry.

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