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PAPER

Pulling on super paramagnetic beads with micro cantilevers: single molecule mechanical assay application

Romina Muñoz[1,3], Felipe Aguilar Sandoval[1], Christian A M Wilson[2] and Francisco Melo[1]

[1] Departamento de Física, Facultad de Ciencia, Universidad de Santiago de Chile, Av. Ecuador 3493, Estación Central, Santiago, Chile
[2] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santos Dumont 964, Independencia, Santiago, Chile
[3] Author to whom any correspondence should be addressed.

E-mail: romina.munoz@usach.cl

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Abstract

This paper demonstrates that it is possible to trap and release a super paramagnetic micro bead by fixing three super paramagnetic micro beads in a triangular array at the sensitive end of a micro cantilever, and by simply switching on/off an external magnetic field. To provide evidence of this principle we trap a micro bead that is attached to the free end of single DNA molecule and that has been previously fixed at the other end to a glass surface, using the standard sample preparation protocol of magnetic tweezers assays. The switching process is reversible which preserves the integrity of the tethered molecule, and a local force applied over the tethered bead excludes the neighbouring beads from the magnetic trap. We have developed a quadrature phase interferometer which is able to perform under fluid environments to accurately measure small deflections, which permits the exploration of DNA elasticity. Our results agree with measurements from magnetic tweezer assays performed under similar conditions. Furthermore, compared to the magnetic tweezer methodology, the combination of the magnetic trap with a suitable measurement system for cantilever deflection, allows for the exploration of a wide range of forces using a local method that has an improved temporal resolution.

Introduction

During diverse molecular processes forces are generated inside cells such as transcription or replication of DNA, protein unfolding, translocation of proteins across membranes, and cell locomotion [1, 2]. At present, due to their versatility, atomic force microscopy, and optical and magnetic tweezers are the most commonly used techniques to measure force at the single molecule level [3]. Optical trapping [4, 5] allows for the application of forces and the manipulation of biomolecules such as DNAs, [6] and the detection of folding and unfolding events of proteins at a single-molecule level [7, 8]. In addition to forces, magnetic tweezers [9] apply torques on micro-magnetic beads linked to single molecules through the displacement and rotation of the external magnets. An advantage of this methodology is that it generates very stable force fields that can be simultaneously applied to many individual molecules within the field of view of the microscope [10]. Of the aforementioned techniques, a disadvantage of magnetic tweezers is that they have the lowest spatial resolution (5–10 nm), but they are suitable for use with very low forces (≈0.1 pN) and do not cause radiation heating or photo damage to the sample. Conversely, micro cantilevers have important advantages with respect to magnetic and optical tweezers since they can be manufactured in a broad range of sensing forces and a variety of systems are available to detect their deflections, which provides high accuracy force assessment over a wide dynamic range [11, 12]. However, micro cantilevers have two important disadvantages for the mechanical assessment of single molecules; firstly, the cantilever tip requires a specific surface functionalization in order to ensure an appropriate and selective linkage to a particular molecule [13], and secondly, following contact with the tip-molecules, several trials and elaborated procedures may be required to ensure that only a single molecule has been linked to the

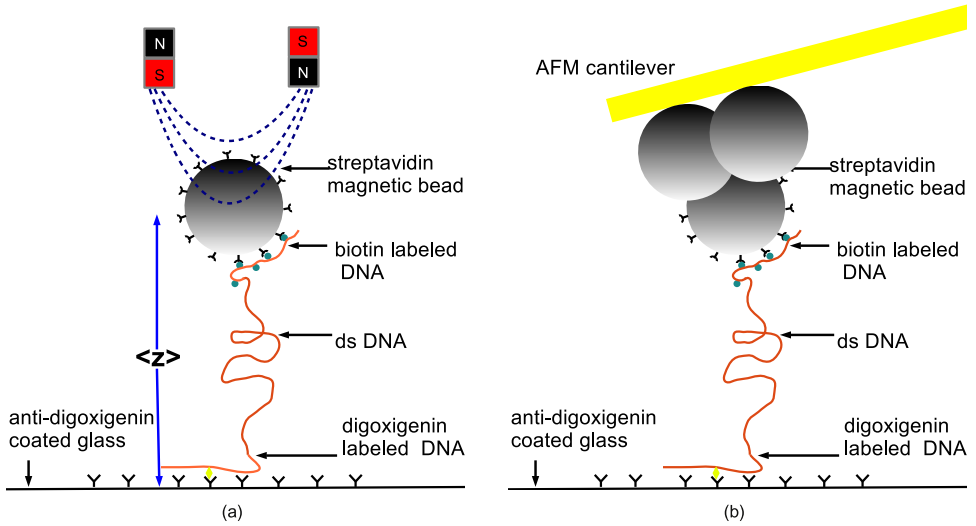

Figure 1. (a) Diagram illustrating tether preparation. One end of a DNA molecule is linked to the glass surface while the other is attached to a super paramagnetic micro bead by surface treatment. (b) Diagram of a micro bead trapped at the end of a cantilever by means of the micro magnetic trap.

cantilever. In addition, once a molecule has been tested, testing another molecule from the sample (with the same cantilever) requires the breaking of the link in order to create a new link, which increases the uncertainty of the operation of subsequent links. This is a serious limitation when a high number of individual molecules are tested and statistical analysis is required, or when the same molecule has to be tested in a non-destructive manner.

We present a magnetic switch suitable to trap and release single tethered super paramagnetic micro beads, without having to unnecessarily submit the neighboring tethered beads to large forces. An example is given in which the switch is implemented at the sensitive end of a micro cantilever, which combines the versatility of cantilever based force measurements with well established methods of sample preparation. We demonstrate that the magnetic switch allows for force spectroscopy measurements on single DNA molecules leading to force extension curves that agree with those obtained through the magnetic tweezer assay.

The methodology for the preparation of tethered molecules is derived from that of magnetic tweezers assays (figure 1(a)), which is briefly discussed in the methods section. In the following sections we demonstrate that the single super paramagnetic bead used in a tether can be captured through the simple act of approaching the magnetic trap and switching on the external magnetic field, as outlined in figure 1(b). Thus, a mechanical assay can be applied, based on the sensing capability of the cantilever, and the tether can be released gradually which prevents any damage to the molecule. As a result this has the benefit of allowing the tracking of specific tethers through the straightforward observation of the micro beads array under an optical microscope.

Materials and methods

Magnetic tweezers experiments

DNA construct for magnetic tweezers

A simple protocol has been developed for the production of half lambda phage DNA, $\lambda/2$ DNA, construct without the use of polymerase chain reaction. It was decided to label one end of the DNA construct with biotin and the other end with digoxigenin, which specifically bind to streptavidin coated beads and to an anti-digoxigenin coated glass surface, respectively. The initial DNA material is 24 μg of λ DNA molecule (NEB, N3011S). The DNA is heated to 65 °C for 5 min in order to expose the cos site, and then rapidly cooled to prevent reannealing. The incorporation of biotin DNA labels (Biotin-14-dCTP, Invitrogen, 19518018) at the cos site is achieved using a Klenow fragment (3'-5' exo-) (NEB, M0212S) and deoxynucleotide (dNTP) solution mix (NEB, N0446S) for 1 h at 37 °C. The reaction is ended by heating to 70 °C for 5 min. In order to obtain single ended biotin functionalized DNA, the molecule is separated into two halves using *XbaI* (NEB, R0145s), this is a restriction enzyme that recognizes TCTAGA sites and cuts the λ DNA molecule into two equal segments ($\lambda/2$). The reaction has a 1 hr duration and occurs at 37 °C. The $\lambda/2$ DNA is filled with digoxigenin DNA labels (digoxigenin-11-dUTP, Roche, 11573152910) using the same Klenow fragment (3'-5' exo-) following the previously described protocol. For optimal results, the ratios of dNTP/digoxigenin or dNTP/biotin should be approximately 25:1. The final solution is passed

through a biospin chromatography column, (Biorad, 7326221) in order to purify the final DNA construct. The estimated length of the final DNA molecule is of 24000 bp or 8.6 μm considering a B-DNA structure.

Binding of DNA to magnetic beads and the glass surface

The tethered molecules are positioned inside a chamber composed of a glass slide and a coverslip (VWR, 1 and 0.17 mm thickness, respectively), with a fixed gap of 100 μm. DNA is linked to the lower glass slide, which is coated with anti-digoxigenin (Roche, 11333089001) following the protocol from Selvin and Ha [14]. The magnetic beads utilized in the experiment are Dynabeads, coated with streptavidin (Life Technologies, M-270, 2.8 μm in diameter). To facilitate DNA glass binding, dual labelled $\lambda/2$ DNA, at 6 pM concentration, is introduced into the chamber and incubated for 5 min at room temperature. Subsequently, beads at a concentration of 30 ng μL^{-1} in 1X PBS buffer at pH = 7.4 are injected into the chamber to promote DNA linkage of the beads. A schematic representation of this step is showed in figure 1(a).

Magnetic tweezers setup

The measurements of force and extension on a single DNA molecule were carried out using a home made magnetic tweezers device. The setup of the device is focused around an inverted microscope equipped with a 100× oil immersed objective of N.A. = 1.3, this permits imaging and analysis of the brownian movement of the beads tethered to the DNA. Images are captured with a USB 2.0 CMOS camera (Thorlabs, DCC1545) with a setting frame rate of 100 fps and a resolution of 80 × 80 pixels (84 nm px^{-1}). The force on the beads is applied through an arrangement of permanent rare Earth magnets. The geometry and spatial location of the magnets was adjusted to optimize the magnetic gradient and improve the force on the beads [15]. The magnitude of the force applied to the beads is controlled through the distance between the beads and the magnets, and the rotation allows for the application of torques on the beads. Displacements and rotation around the axis of symmetry are controlled by stepper motors (Physik Instrumente, PI M-16 and PRS-200). A piezoelectric objective actuator (Physik Instrumente, P-720) allows for precise motion of the objective for the calibration step. Calibration consists of capturing the diffraction patterns of a bead at different distances from the reference surface. This is done by moving the objective at intervals of 100 nm throughout a total distance of 20 μm. The position of the bead during the pulling test is then obtained by a straightforward comparison of the actual bead diffraction pattern with examples from the patterns library, which is generated through the calibration.

Magnetic trap

Magnetic trap theory

A simplified operation of our magnetic switching trap is illustrated in figure 2. The magnetic trap consists of three super paramagnetic micro beads with centers forming the vertex of an equilateral triangle. Beads are fixed at the free end of the cantilever. In the presence of a constant external magnetic field, beads are magnetized and cause strong field gradients in neighboring regions. Therefore, if a fourth super paramagnetic bead, polarized by the external field, approaches the trap from a sufficiently short distance, it experiences attractive dipolar forces. In order to provide an estimate of the force applied to a bead by the trap, we consider as a working hypothesis that the external field $\vec{B}_0$ is homogenous within close proximity to the trap and is responsible for inducing a magnetization on each bead within the trap. To mathematically describe our magnetic system, we approximate each bead to a magnetic dipole whose moment is proportional to the external field. In addition, we neglect any magnetic influence of the cantilever. Thus, $\vec{m}_b = V_b\chi_b\vec{B}_0/\mu_0$, where χ_b is the magnetic susceptibility and V_b is the volume of the super paramagnetic bead. The force acting on the active bead is then,

$$\vec{F} = \nabla\left(\vec{m}_b \cdot \vec{B_T}\right), \tag{1}$$

where $\vec{B_T} = \vec{B}_0 + \vec{B}_t$ is the total magnetic field, which we have approximated as the linear superposition of the external field plus the field produced by the induced dipole moments on the trap $\vec{B}_t$. Since the external field is nearly homogeneous and directed along the vertical $\hat{z}$, equation (1) becomes,

$$\vec{F} = \frac{V_b\chi_b B_0}{\mu_0}\nabla\left(\hat{z} \cdot \vec{B}_t\right), \tag{2}$$

which provides an estimate of the vertical magnetic force exerted by the trap on the active bead located at the point of coordinates (x, y, z) as,

$$F_z = \frac{V_b\chi_b B_0}{\mu_0}\frac{\partial}{\partial z}B_{t,z}(x, y, z). \tag{3}$$

The magnetic field provided by the trap is approximated to that of three magnetic induced dipoles ($\vec{m}_i$, with i = 1 to 3) arranged in an equilateral triangle. Thus, the trap field reads,

$$\vec{B}_t = \frac{\mu_0}{4\pi}\left[\sum_{i=1}^{3}\left(\frac{3\vec{r}_i\left(\vec{m}_i \cdot \vec{r}_i\right)}{r_i^5} - \frac{\vec{m}_i}{r_i^3}\right)\right], \tag{4}$$

where $\vec{r}_i = \vec{r} - \vec{a}_i$, while $\vec{r}$ describes the point in space and $\vec{a}_i$ indicates the vertex of the equilateral triangle with a base equal to the beads diameter, defined by the center of the beads with radius R and $|\vec{a}_i| = a = 2R/\sqrt{3}$. Since the external field is along $\hat{z}$ and the micro beads are assumed to have equal properties, the induced dipole moments can be written as, $\vec{m}_i = V_b\chi_b B_0/\mu_0\ \hat{z}$. After replacing m_i in equation (4) and then B_t in equation (3), the vertical

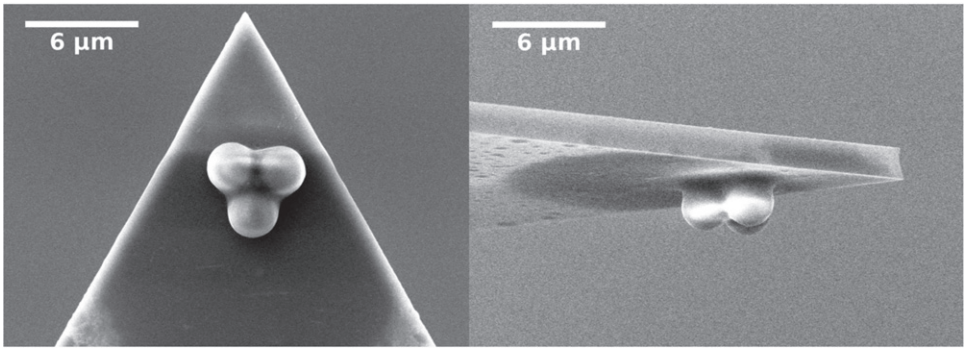

Figure 2. Scanning electron microscope images illustrating top and side views of the cantilever end modified by the inclusion of three super paramagnetic beads forming the magnetic trap.

force on the active beads located at a distance z, along the axis of symmetry $(x,\ y = 0)$ reads,

$$F_z(z)|_{x,y=0} = \frac{3V_b^2\chi_b^2 B_0^2}{4\pi\mu_0}\frac{z}{\left(z^2+a^2\right)^{5/2}}\frac{9a^2-6z^2}{z^2+a^2}. \quad (5)$$

The closest approach distance for the active bead in the trap is $z_{\min} = \sqrt{\frac{2}{3}}2R$. The magnetic force at contact then becomes,

$$F_z(z = z_{\min})|_{x,y=0} = -\frac{\sqrt{6}}{64}\frac{V_b^2\chi_b^2 B_0^2}{\pi\mu_0 R^4}. \quad (6)$$

In this case (Dynabeads) R = 1.4 μm, $\chi_b = 0.76$, $B_0 \approx 0.03$ T, $\mu_0 = 4\pi \times 10^{-7}$ TmA^{-1}. With these values, the maximum force applied to the trap can easily exceed 100 pN through an increase in the external field. Considering the force for DNA overstretching is about 65 pN, the magnetic trap provides a wide range of magnetic forces for DNA assessment.

Magnetic trap preparation protocol

Microspheres, 2.8 μm diameter, streptavidin coated Dynabeads, are diluted in milliQ water at concentrations of 50 ng μL^{-1}. A 10 μL volume of the resulting solution is deposited onto a glass slide and left exposed to the atmosphere to dry. To eliminate salt traces, micro beads are further rinsed with distilled water, however as a result of this process some micro beads will be lost. Under an inverted optical microscope (Nikon Eclipse), a tipless micro cantilever with nominal force constant 0.03 N m^{-1} (Arrow-TL1 from Nano World), previously attached to the arm of a three-axis water hydraulic micromanipulator with a minimum graduation of 0.2 μm (MHW-3, from Narishige), facilitates some micro beads to group into triangular clusters of three particles. Each micro bead is separated from one another by a sufficient distance to allow for their manipulation without interference (about 100 μm). Slow curing epoxy adhesive (Araldit) provides strong attachment of clusters to the cantilevers and allows enough time for manual positioning. To avoid an excess of adhesive, a thin layer of epoxy is prepared onto the glass slide a few millimeters distance from the cluster area. The sensitive end of a tipless cantilever is brought close to the adhesive until contact is made and some adhesive is transferred to the surface by capillarity action. The cantilever is then gently pushed against the selected cluster (see figure 2). Prior to use the cantilever is left to dry at room temperature for several hours.

Binding of DNA to magnetic beads and the glass surface

The fluid cell is an open reservoir consisting of a glass surface (coverslip 0.15 mm thick) and a small glass tube with internal diameter of 19 mm and wall thickness of 1 mm, which is fixed at the center of the glass surface by a small amount of UV optical glue. The cell is initially rinsed with milliQ water followed by abundant ethanol and dried with nitrogen flow. The glass surface is coated with anti-digoxigenin following the same protocol as in the magnetic tweezers experiment. The biotinylated DNA in PBS buffer is put inside the glass surface of the fluid cell and incubated for 5 min at room temperature. Subsequently the same Dynabeads in PBS buffer are put with the DNA inside of the fluid cell, resulting in a final DNA concentration of 6 pM, and 30 ng μL^{-1} of beads. The solution is kept at room temperature and under a gentle rotation for 15 min to promote homogeneous linking.

Magnetic trap setup

A diagram of the experimental set up is presented in figure 3. Observations are performed with a 40× microscope objective positioned in an inverted position, and images of tethered beads are captured with a Thorlabs USB camera, model DCC1645C, with a resolution of 1280 × 1024 pixels.

A coil positioned concentrically to the fluid cell, generates a small homogeneous magnetic field in the area of interest. This can be increased or decreased by varying the current within the coil, allowing for either the trapping or releasing of a tethered bead. An electric circuit manages the variation of the current and

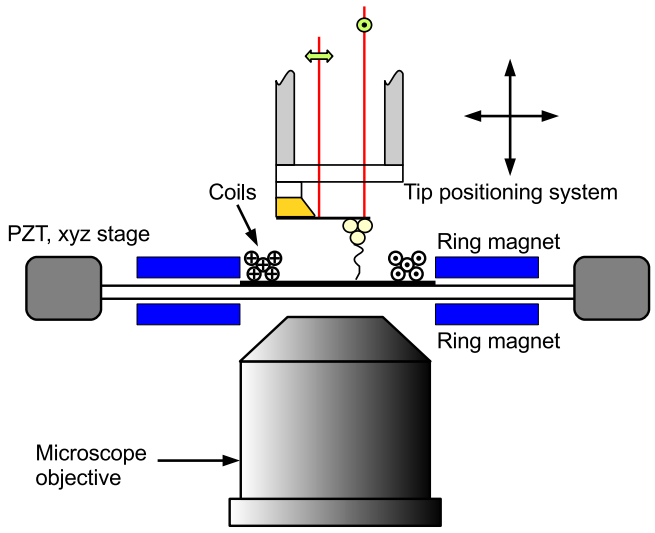

Figure 3. Diagram representing the experimental set-up of the cantilever based force spectroscopy. A coil provides a homogeneous magnetic field, which permits for either the trapping or releasing of a tethered bead. A micro positioning system permits the cantilever tip to be positioned onto the selected tethered bead. Two polarized parallel lasers beams impinge upon the sensitive cantilever part and the cantilever body. After reflection, the phase difference is detected by a quadrature phase interferometer, which provides the cantilever deflection. As an optional alternative, it is possible to use a pair of ring magnets in order to avoid the overheating of the coil from long periods of use, this option restricts the coil use for only disconnecting the trapped bead.

triggers the camera to capture images. The magnetic field is measured with a magnetic field sensor (sensitivity 9×10^{-4} G).

Cantilever deflection measurement

A variety of well known methods can be used to determine the force exerted on the cantilever [16, 17]. In this situation, to reduce errors and increase sensitivity, a interferometric system is implemented to accurately detect the absolute deflection of the cantilever maintaining continual sensitivity and low force noise. The key element of this device is the quadrature phase interferometer, as described in [18], in this case its capabilities have been extended to perform under fluid environments. Essentially, two parallel beams at approximately 700 μm apart impinge simultaneously on the bendable part of the cantilever and its rigid base. The beams are of perpendicular relative polarization and are obtained from a He-Ne single beam by means of a calcite beam displacer. Thus, the cantilever deflection induces a phase difference, ϕ, in the reflected beams, which is detected as a contrast of intensities at two pairs of photodiodes [19]. The contrast in one pair is $\cos(\phi)$, and in the other pair it is $\sin(\phi)$ due to the presence of the $\lambda/4$ plate. Thus, in a polar representation, the contrast describes a circle of constant radius, which ensures constant sensitivity over the whole domain ϕ and of the cantilever deflection (see figure 3). Losses from reflection beams are minimized by using an anti-reflex window which also serves as the upper lid of the fluid cell. In addition, a spacer of 1 mm thickness allows the cantilever to be fixed parallel to the lower face of this window.

Results and discussion

Magnetic force on a single super paramagnetic bead, magnetic trap characterization

To characterize the magnetic click, we primarily investigate the force it exerts on a microsphere fixed onto the bottom of a glass cell, as a function of the vertical distance. In order to simplify the process, in the absence of magnetic field, we locate the magnetic trap just above the bead at a distance of about 6 μm. The magnetic interaction is measured through the detection of cantilever deflection and carried out in a fluid environment. The measurement is carried out by generating cycles of current, which in turn produces cycles of a magnetic field (of about 200 G amplitude for 0.2 s and 0 G for 0.8 s), simultaneously the cantilever approaches the bead at a constant velocity of 100 nm s^{-1}. The magnetic force is then modulated at the same frequency (figure 4(a)), which allows for the extraction the extend of thermal drift on the cantilever detection caused by the heat of the coil. Thus, the magnetic force is then calculated by the envelope of the cantilever deflexion multiplied by the cantilever stiffness. In figure 4(b) the resulting force is contrasted against the predictions obtained from equation (5). The solid line represents the best fit using the magnetic field as a fitting parameter. Equation (5) provides a satisfactory explanation for the magnetic force, however a B_0 value that is approximately 30% higher than the experimental value is required to obtain a good agreement, indicating that equation (5) underestimates the magnetic force.

DNA stretching by magnetic click

In order to trap a tethered molecule, primarily, in the absence of a magnetic field the trap is directly

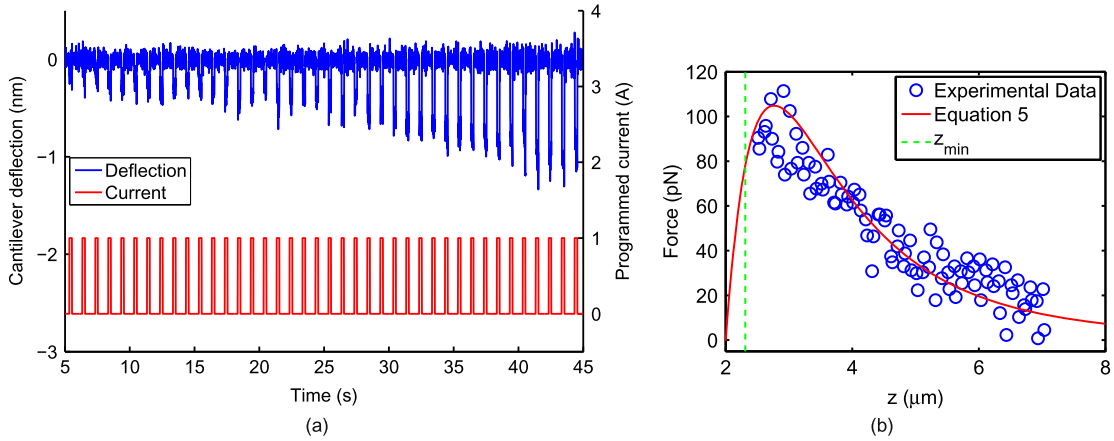

Figure 4. (a) Cantilever deflection induced by magnetic field switching as the magnetic click approaches the super paramagnetic bead fixed on the glass surface. (b) Magnetic force obtained from the envelope of cantilever deflection as a function of the distance from the fixed bead. The green vertical line indicates the minimum distance at which it is possible for the bead to enter the trap due to the steric effect. The solid line is the best fit obtained from equation (5).

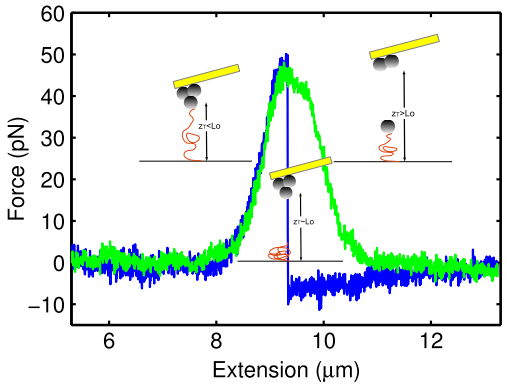

Figure 5. Typical force extension curve for a DNA tethered molecule obtained through the measurement of the cantilever deflection. Green curve: at sufficient high force and low magnetic field the magnetic bead detaches from the trap. Blue curve: occasionally DNA breaks, leading to an abrupt drop in the pulling force to zero.

positioned above the selected bead at a distance of less than 1 μm. The magnetic field is then switched on, which induces the bead to lodge into the cavity of the trap. It is found that a field of 80 G is both sufficient and avoids over heating. Force curves are then obtained through the vertical displacement of the sample, which is achieved by means of the high performance piezoelectric stage. figure 5 presents the force exerted by the cantilever on the DNA tethered molecule as function of the DNA extension, this was determined by measuring the distance from the bottom cell to the location of the tethered bead in the magnetic trap. The force rapidly increases as the DNA molecule stretches and approaches an extension close to its contour length. However, at relatively low B_0 ($\approx 80G$) and sufficiently high DNA stress (in this case approximately 50 pN), the tethered bead can detach from the magnetic click. After detachment, the DNA molecule retracts (see rightmost inset of figure 5) and this during this process the overall magnetic force is not equal to the DNA tension; thus the DNA almost completely retracts (green curve). Occasionally DNA may rupture as depicted on the middle inset of figure 5, this process is denoted by the pulling force abruptly dropping to zero (blue curve). The rupture of DNA at low forces can be explained by the biochemistry of the $\lambda/2$ DNA synthesis, which implies that the linkage of DNA labels relies on a reduced number of biotin or digoxigenin molecules.

In order to test the consistency of the force measurements, we apply the worm-like chain model (WLC) to the force data obtained by the cantilever click. In this model, the force reads [20]

$$F = \frac{k_B T}{L_p} \times \left[\frac{1}{4\left(1 - \frac{<z>}{L_0}\right)^2} + \frac{<z>}{L_0} - \frac{1}{4} + \sum_{i=2}^{i=7} a_i \left(\frac{<z>}{L_0} \right)^i \right], \quad (7)$$

where L_p is the persistence length, L_0 the contour length and $<z>$ the extension. With, $a_2 = -0.5164228$, $a_3 = -2.737418$, $a_4 = -16.07497$, $a_5 = -38.87607$, $a_6 = -39.49944$ and $a_7 = -14.17718$.

The WLC model fits our data at low forces but deviates significantly at higher extensions, figure 6(a). This behavior has been reported in previous work [21] and is due to the finite stretching modulus of real DNA, which is not included in the WLC (equation (7)). Also, force data are contrasted with direct measurements, which are obtained during the magnetic tweezers technique, figure 6(b). Comparisons indicate that the magnetic click provides force data that are very similar to that obtained with the

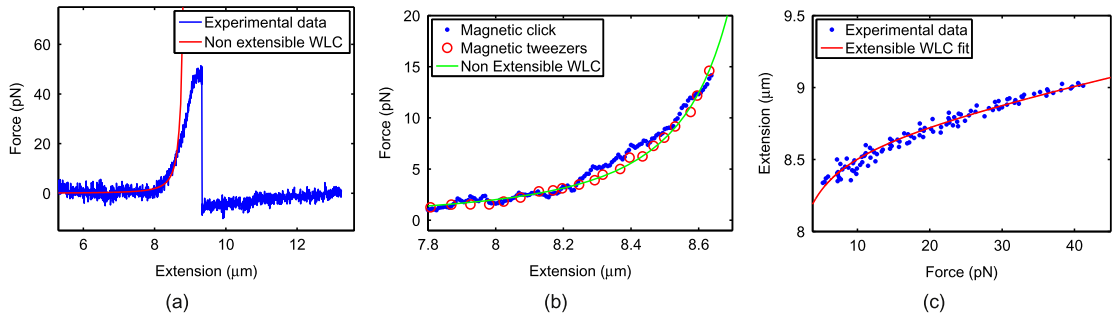

Figure 6. (a) Typical force curve obtained with the magnetic click methodology. The solid line is the fit applying the WLC model, equation (7). Agreement is only obtained at low force with fit parameters $L_0 = 8.9 \pm 1.0\,\mu$m and $L_p = 48 \pm 7$ nm. (b) Comparison of the force versus extension curves obtained through the magnetic tweezer and the cantilever click in the low force regime, showing the same fit to the magnetic tweezers data with parameters $L_0 = 9.0 \pm 0.9\,\mu$m and $L_p = 46 \pm 7$ nm. (c) Extension versus force curve fitted in the high force regime by using the DNA extensible model, equation (8). With fit parameters $L_0 = 8.8 \pm 0.8\,\mu$m, $L_p = 49 \pm 5$ nm and $K_0 = 864$ pN.

established magnetic tweezers method. Additionally, in both cases the adjustment of the WLC model leads to values of $L_p \approx 50$ nm (obtained as fitting parameter), that are consistent with the accepted values [20, 22]. This indicates that DNA preserves its integrity during the magnetic click assay.

The extensible WLC model can be applied to account for the behavior of DNA at greater extension. Besides the entropic elasticity, this model accounts for the enthalpy contribution to the DNA stiffness. In this model the extension $\langle z \rangle$ is expressed as a function of the force, F as,

$$\langle z \rangle = L_0 \left(1 - \frac{1}{2} \left(\frac{k_B T}{F L_p} \right)^{1/2} + \frac{F}{K_0} \right), \qquad (8)$$

where K_0 is the stretch modulus.

A satisfactory agreement is observed (figure 6(c)), leading to DNA extension modulus values ($K_0 = 864$ pN) which are close to the well accepted values [21], supporting our magnetic click method.

Conclusions

In conclusion, by developing a magnetic trap at the bendable end of a microcantilever, we have shown the potential for a hybrid technique which combines the advantages of magnetic tweezer assays with cantilever based force measurements. The main principles described here can be further developed to miniaturize and automatize the aforementioned hybrid device. For example, the experiments showed that a small and easily produced magnetic field generated by a small electrical current in the coil, used in conjunction with the micro beads, is sufficient to induce the trapping effect. Thus, if enough space is available between the lens and the fluid cell, it should be possible to implement two coils in the Helmholtz's configuration to improve the magnetic field homogeneity. In order to measure the deflection of the cantilever, several available solutions can be implemented, dependent on application, based on laser beam deflection or interferometry. In this case, our choice has shown that it is possible to obtain reliable DNA force curves, even at very low forces. However, cantilevers provide a wide range of forces, which open future opportunities to explore the mechanical properties of biological systems with a broader scope and better temporal resolution.

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References

[1] Bustamante C, Chemla Y, Forde N and Izhaky D 2004 Mechanical processes in biochemistry *Annu. Rev. Biochem.* **73** 705–48
[2] Bustamante C, Kaiser C, Maillard R, Goldman D and Wilson C A M 2014 Mechanisms of cellular proteostasis: insights from single-molecule approaches *Annu. Rev. Biophys.* **43** 119–40
[3] Deniz A, Mukhopadhyay S and Lemke E A 2008 Single-molecule biophysics: at the interface of biology, physics and chemistry *J. R. Soc. Interface* **5** 15–45
[4] Ashkin A 1970 Acceleration and trapping of particles by radiation pressure *Phys. Rev. Lett.* **24** 156
[5] Ashkin A, Dziedzic J M, Bjorkholm J E and Chu S 1986 Observation of a single-beam gradient force optical trap for dielectric particles *Opt. Lett.* **11** 288–90
[6] Smith S B, Cui Y and Bustamante C 2003 Optical-trap force transducer that operates by direct measurement of light momentum *Methods Enzymol.* **361** 134–62
[7] Bustamante C, Cheng W and Mejia Y X 2011 Revisiting the central dogma one molecule at a time *Cell* **144** 480–97
[8] Shank E, Cecconi C, Dill J, Marqusee S and Bustamante C 2010 The folding cooperativity of a protein is controlled by its chain topology *Nature* **465** 637–40

[9] Strick T, Allemand J F, Croquette V and Bensimon D 2000 Twisting and stretching single DNA molecules *Prog. Biophys. Mol. Biol.* **74** 115–40
[10] de Vlaminck I, Henighan T, van Loenhout M T J, Burnham D R and Dekker C 2012 Magnetic forces and DNA mechanics in multiplexed magnetic tweezers *PloS One* **7** e41432
[11] Viani M B, Schäffer T E, Chand A, Rief M, Gaub H E and Hansma P K 1999 Small cantilevers for force spectroscopy of single molecules *J. Appl. Phys.* **86** 2258–62
[12] Marshall B T, Sarangapani K K, Wu J, Lawrence M B, McEver R P and Zhu C 2006 Measuring molecular elasticity by atomic force microscope cantilever fluctuations *Biophys. J.* **90** 681–92
[13] Strunz T, Oroszlan K, Schäfer R and Güntherodt H J 1999 Dynamic force spectroscopy of single DNA molecules *Proc. Natl Acad. Sci. USA* **96** 11277–82
[14] Lionnet T, Allemand J F, Revyakin A, Strick T R, Saleh O A, Bensimon D and Croquette V 2008 Single-molecule studies using magnetic traps *Single-Molecule Techniques, a Laboratory Manual* ed P R Selvin and T Ha (New York: Cold Spring Harbor Laboratory) pp 366–7
[15] Humphries D E, Hong S C, Cozzarelli N R, Cozzarelli L A and Pollard M J 2009 Hybrid magnet devices for molecule manipulation and small scale high gradient field applications *Patent* 7474184
[16] Binnig G, Quate C F and Gerber Ch 1986 Atomic force microscope *Phys. Rev. Lett.* **56** 930
[17] Vashist S K 2007 A review of microcantilevers for sensing applications *J. Nanotechnology* **3** 1–18
[18] Paolino P, Aguilar Sandoval F and Bellon L 2013 Quadrature phase interferometer for high resolution force spectroscopy *Rev. Sci. Instrum.* **84** 095001
[19] Bellon L, Ciliberto S, Boubaker H and Guyon L 2002 Differential interferometry with a complex contrast *Opt. Commun.* **207** 49–56
[20] Bouchiat C, Wang M D, Allemand J F, Strick T, Block S M and Croquette V 1999 Estimating the persistence length of a worm-like chain molecule from force-extension measurements *Biophys. J.* **76** 409–13
[21] Smith S B, Cui Y and Bustamante C 1996 Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules *Science* **271** 795–9
[22] Baumann C G, Smith S B, Bloomfield V A and Bustamante C 1997 Ionic effects on the elasticity of single DNA molecules *Proc. Natl Acad. Sci. USA* **94** 6185–90