Magnetic Force Probe for Nanoscale Biomolecules

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We present a new technique to measure the mechanical properties of small biomolecules. This technique uses long range repulsive colloidal forces together with magnetic attraction as a force probing tool. The biomolecules are grafted between superparamagnetic particles, which are regularly spaced within long chains maintained by an external magnetic field. Varying the magnetic field results in compression or extension of the molecules between the particles. In order to demonstrate this technique we use, as a size controlled model molecule, a short double stranded DNA (151 base pairs) for which the force-extension law is determined and found in agreement with existing predictions.

During the last decade, forces and stresses that molecules exert on each other have become directly measurable using various single molecule techniques [1–3]. It has become clear that piconewton forces are controlling many key processes in biology, involving receptor-ligand pairs, protein and nucleic acid structures, or identical self-assembled proteins. For instance, single molecule measurements have provided significant understanding of biopolymers longer than their persistence length [4–6]. By contrast, for small complexes or for biopolymers smaller than their persistence length, there are only a few data reporting on their mechanical properties. Indeed, most of the single molecule techniques do not apply, except the atomic force microscope (AFM) which is particularly suited to measure large stretching forces [7–10]. However, to our knowledge, no technique addresses the regime of small reversible deformations in the vicinity of unstressed conditions.

In this Letter, we use the magnetic chaining technique (MCT) to investigate the elastic force-extension law of nanoscale biocomplexes in the domain of small deformations, with a force resolution of 0.1 pN and a distance resolution of 1 to 2 nm. Using magnetic colloids, we apply a compression or extension force to the biocomplexes. When a magnetic field is applied, magnetic colloids spontaneously self-organize into linear chains. As a result of the equilibrium between attractive magnetic and repulsive colloidal forces, monodisperse particles are regularly spaced within these chains. The measurement of the particles spacing and the subsequent computation of the magnetic force allow, by varying the magnetic field, to reconstruct colloidal force-distance profiles [11,12]. Furthermore, when small biocomplexes are inserted between magnetic particles, they generate additional elastic forces that can be probed by the above mentioned attractive and repulsive colloidal forces.

More precisely, to measure the force-extension law of a small biomolecule, we employ a two steps strategy. First,

the background repulsive force-distance profile, in the absence of biomolecules, $F_{bg}(h)$, is measured. Then, once the biomolecules have been properly attached within each interval between colloids, the same measurement is repeated allowing to determine the force-distance profile of this irreversible assembly $F_{ir}(h)$. The force $F_{ir}(h)$ includes two contributions, one being the repulsive background and the other originating from the presence of the linker. In the limit of low grafting densities, these two forces must be additive. Then, from substracting $F_{bg}(h)$ from $F_{ir}(h)$ we can compute the force-extension profile of the linkers $F_l(h)$. Finally, from the number of links per particle we deduce the force-extension law of a single complex assuming biomolecules behave like springs in parallel. In order to demonstrate this technique we use, as a size controlled model molecule, a short double stranded DNA (151 base pairs) for which the force-extension law has been predicted. Superparamagnetic particles with a diameter of about 200 nm and a polydispersity of about 18% in volume are purchased from Ademtech [13]. The particles are first size fractionated using a magnetically induced phase separation. A sample of average diameter 176 nm is isolated with a polydispersity of 5% in volume [14]. Then, this sample is covalently grafted with streptavidin [15]. A number of 185 available binding sites per bead is measured by inverse titration using fluorescein biotin [16]. As one of the most important requirements, this colloidal dispersion is stable and does not aggregate during a storage period of several months or under magnetic field. Indeed, they redisperse immediately once the field is switched off. This property is ensured by the presence of the repulsive colloidal forces which also guarantees the absence of any unspecific aggregation. We present in Fig. 1 this colloidal force-distance background, at four different ionic strengths, measured with the MCT as follows. Under magnetic field, the particles are regularly spaced along chains parallel to the magnetic field. Therefore the chains, illuminated in the direction of the field, diffract light. We collect
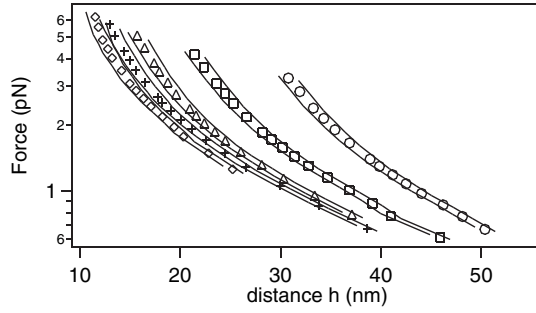
FIG. 1.   Force-distance profile of the particles grafted with
streptavidin, $C_p = 0.05\%$ volume in pH 7.2 phosphate buffer
as a function of the ionic strength. ○: 1 mM ($\kappa^{-1} = 9.5$ nm), □:
2 mM ($\kappa^{-1} = 6.7$ nm), △: 5 mM ($\kappa^{-1} = 4.2$ nm), +: 10 mM
($\kappa^{-1} = 3$ nm), ◇: 20 mM ($\kappa^{-1} = 2.1$ nm). The surface to
surface separation h is deduced from: $h = d - 2R$. The distance
d is measured from Bragg scattering and $2R$ is the hard core
diameter. Plain lines give the confidence interval for the calcu-
lated profiles, taking into account an error of 1 nm on the
distance measured. To measure R, the particles were pushed to
contact in a solution of 10 mM chlorhydric acid for which the
COOH functions present at the surface of the particles are
completely protonated. The evolution of the repulsive profiles
with ionic strength shows that both electrostatic and steric forces
help to stabilize the particles.

the diffracted light in the backscattering direction and
measure its intensity as a function of the wavelength (using
a Avaspec-2048 spectrometer). According to Bragg's law,
this intensity exhibits a maximum at $\lambda = 2nd$, d being the
center-to-center distance between adjacent colloids and n
the refractive index of water. We then compute the attrac-
tive magnetic force, F_{magn}, between neighboring colloids,
according to [17]

$$F_{\text{magn}} = -\frac{1.202\mu_0}{2\pi}\frac{3m^2}{d^4}, \qquad (1)$$

where μ_0 is the magnetic permeability of free space and m
is the induced magnetic moment of each particle [18]. Let
us now state that the particles are at mechanical equilib-
rium. The repulsive colloidal force, F_{rep}, is then equal to
the attractive magnetic force. Measuring the center-to-
center distance at various magnetic field amplitudes, rang-
ing from 10 to 80 mT, we can now build the force-distance
profile, $F_{\text{rep}}(d)$.

To validate our force probe method, we use DNA as a
well described biomolecule. Dibiotin ds-DNA of 151 bp
was synthesized by polymerase chain reaction (PCR) using
biotinylated primers and a λ-DNA template. Grafted col-
loidal particles and ds-DNA are mixed in a pH 7.2 phos-
phate buffer at a final concentration of $Cp = 1.3 \times 10^{11}$
particles per mL and $C_{\text{DNA}} = 2.7 \times 10^{12}$ to 7.3×10^{12}
molecules per mL, respectively. The sample is then incu-
bated at room temperature for 10 minutes to allow for the
coupling of DNA onto the particles. A magnetic field of

80 mT is finally applied for 240 s. As a first demonstration
of the controlled formation of permanent bounds between
particles, we perform two distinct experiments, one with
dibiotin ds-DNA, and a control one with monobiotin ds-
DNA. In each case, we turned the field off after 240 s, and
directly observed the chains under the microscope (Fig. 2).
As expected, only the dibiotin ds-DNA which has biotin at
each end is able to bridge particles. After links are formed
(240 s at 80 mT), the force-distance profile is systemati-
cally measured by applying 5 times the same protocol: the
field is decreased from 80 to 10 mT, and then increased
again from 10 to 80 mT. The ten different profiles arising
from these ten repeated ramps are shown in Fig. 3. The data
all superimpose on a single set, which confirms that only a
reversible compression/extension of the engaged links is
probed and that no additional links are formed during these
ramps. In our procedure, we will further average over all
these force-distance profiles. In the very low force regime,
when magnetic interactions become comparable to kT, the
chains persist because particles are linked together but
their local orientation relative to the direction of observa-
tion becomes less pronounced. This leads to a reduction of
the average measured particle separation. This experimen-
tal artifact sets the lower limit of the force detection in our
technique.

The background $F_{\text{bg}}(h)$ and the irreversible chain profile
$F_{\text{ir}}(h)$, as well as the substraction of one from the other,
which represents the "linker contribution" $F_l(h)$, are
shown in Fig. 4. $F_{\text{ir}}(h)$ crosses $F_{\text{bg}}(h)$ at a particular
separation h_0 for which there is no load on the ds-DNA.
This crossover demonstrates that this technique can ex-
plore both the regimes of extension and compression:
magnetic attractive forces are used to probe the compres-
sion regime while the repulsive colloidal ones are used to
explore the extension regime. The force profile $F_l(h)$ is
shown for various concentrations of ds-DNA in the inset of
Fig. 4. The slope of $F_l(h)$ increases with the number of
links, which reflects that the contribution of each DNA is
additive. The intercept with the x axis (h_0) remains essen-
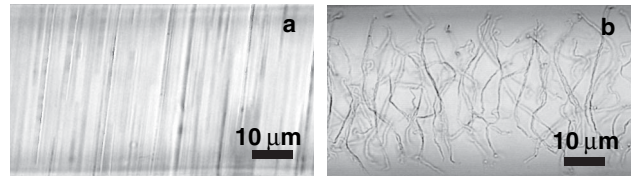tially constant, within a 1 to 2 nm experimental error.

FIG. 2.   Formation of permanent chains under optical micro-
scope. (a) Under magnetic field (80 mT), the particles align in
the direction of the field due to dipolar interactions; (b) 2 min
after removing the magnetic field, the chains remain permanent
due to bridging by ds-DNA. The filaments have lost their orien-
tation as a result of Brownian motion and flexibility [22]. $C_p =
1.3 \times 10^{11}$ particles/mL and $C_{\text{DNA}} = 7.3 \times 10^{12}$ molecules/mL.
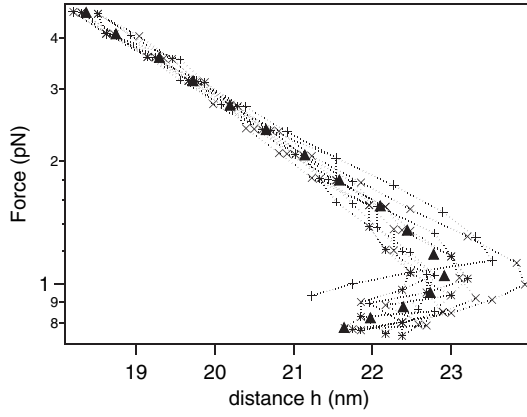
FIG. 3. Force-distance profiles in the presence of 1.9 DNA on average between adjacent particles, in phosphate buffer 10 mM pH 7.2. +: increasing field ramps, ×: decreasing field ramps, ▲: average force-distance profile. Dashed lines are guides to the eye.

We model $F_l(h)$ by accounting for (i) the averaged number of DNA molecules involved within each interspace, (ii) the statistical distribution of their tilt angle φ relative to the chain axis, and (iii) the intrinsic force-extension law for a single DNA. For these short ds-DNA, we use an expression initially derivated for actin filaments that concerns semiflexible biopolymers in the regime where their contour length L_c is less than or equal to their persistence length L_p [19]:

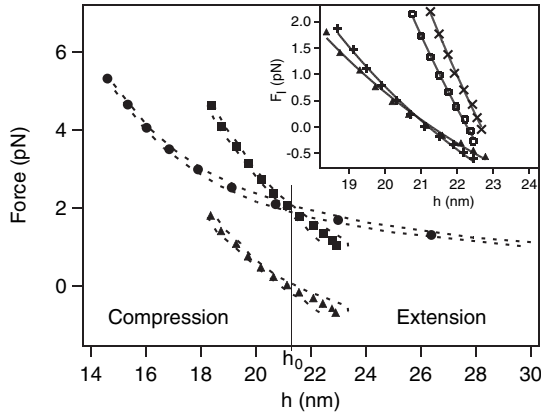$$F = -90k_BTL_p^2\frac{L-L_0}{L^4} \quad (2)$$

FIG. 4. Comparison between average force-distance profiles in the absence and in the presence of 1.9 DNA on average between adjacent particles, in phosphate buffer 10 mM pH 7.2: ●: F_{Bg}, ■: F_{irr}, ▲: $F_l = F_{irr} - F_{Bg}$. Dashed lines correspond to the error on force-distance profiles, calculated from the standard deviation of the measured profiles. Inset: contribution of the linker F_l and fit of the data for various numbers of DNA between the particles. ▲: 1.9 DNA, +: 3 DNA, □: 4.7 DNA, ×: 5.5 DNA. Plain lines: fit of the data with $F_{tot} = N\langle F\rangle_\varphi$, with $N = 1.9$ and $\langle F\rangle_\varphi$ given by Eq. (3).

with L the actual length of the link and L_0 its length at rest. By contrast with the Hooke regime valid for L much larger than L_p, this scaling law reflects that the fraction of the excess polymer length in the form of thermal undulation decreases as L is reduced, leading to less contour available to "pull out" under the applied stress. N is computed on the basis of a homogeneously distributed specific adsorption of the total amount of DNA onto the particles. As shown in Fig. 5, we assume that the DNA molecules that participate to the linkage are located on the portions of sphere facing each other (shattered surface). Looping of the DNA due to reaction of both its extremities on the same bead is considered negligible. Indeed, assuming that the links form independently from one another, their distribution follows a Poisson law and the mean chain length is 2.7 for $N = 1$ and 6.7 for $N = 1.9$. Microscopic observations reveal that chains at least as long as 7 particles form for $N = 1.9$, ruling out the hypothesis of DNA looping. F_l is thus given by

$$F_l = -N\left\langle 90k_BTL_p^2\frac{L-L_0}{L^4}\right\rangle_\varphi \quad (3)$$

with the averaging over φ detailed in [20]. In this expression, only the parameters L_p and L_0 are unknown and can then be determined from fitting our data with Eq. (3) as shown in the inset of Fig. 4. The fitted values of L_p and L_0 as a function of N are reported in Table I, for N varying from almost 2 to 5. We find for each case a value for L_p very close to the 50 nm value given by measurements on longer DNA molecules [4–6]. We also find a good agreement for L_0, which is predicted to be 86% of the contour length, i.e., 44 nm for 151 bp fragments [21].
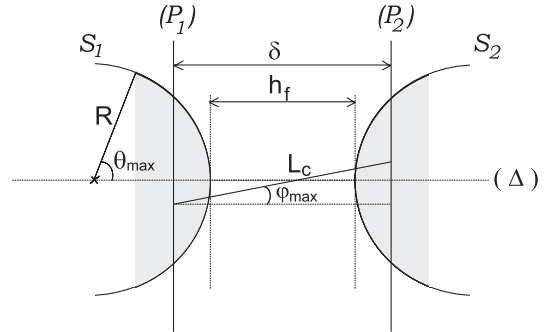

FIG. 5. Definition of the different geometrical parameters appearing in the model. The angle θ_{max} defines the reactive portion of sphere and the distance h_f is the distance between the particles during the linking process (formation of permanent chains): $\cos\theta_{max} = (h_f + 2R - L_c)/2R$. $L_c = 51$ nm is the contour length of the DNA and $R = 88$ nm is the radius of the particles. δ is the distance between the planes (P_1) and (P_2) that are used for approximation. φ_{max} defines the maximal orientation that can take a DNA molecule when linking the beads. The figure is invariant by rotation around ($\triangle$), the axis of the chain.

TABLE I. Results obtained from the fit of the data in Fig. 4 with only two free parameters: L_p, the DNA persistence length and L_0, the 151 bp fragment length at rest. The given errors correspond to the standard deviation of the experimental points to the fit.

DNA number	L_p (nm)	L_0 (nm)
1.9	45.6 ± 1.0	39.7 ± 0.1
3.0	40.0 ± 0.9	39.7 ± 0.1
4.7	48.6 ± 1.2	40.6 ± 0.1
5.5	48.6 ± 1.2	41.0 ± 0.1

In this Letter we have demonstrated the possibility of measuring the mechanical properties of small biocomplexes by grafting them in between magnetic colloids and using the MCT. We used a short ds-DNA as a first model molecule and confirmed the validity of its force-extension prediction.

Whereas mechanical response of DNA molecules much longer than the persistence length may be studied by optical and magnetic traps [4,6], mechanical properties of short ds-DNA molecules are difficult to measure. Our technique complements AFM measurements. Indeed, AFM has been used to apply high extension forces to ds-DNA molecules [8], whereas MCT allows to reach the regime of small deformations, both in compression and extension.

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$$\langle F \rangle_\varphi = -\frac{90 k_B T L_p^2}{2\varphi_{max}} \int_{-\varphi_{max}}^{\varphi_{max}} \left(\frac{1}{\delta^3}\cos^4\varphi - \frac{L_0}{\delta^4}\cos^5\varphi \right) d\varphi$$

and after integration:

$$\langle F \rangle_\varphi = -\frac{90 k_B T L_p^2}{\varphi_{max}}$$
$$\times \left[\frac{1}{\delta^3}\left(\frac{3}{8}\varphi_{max} + \frac{1}{4}\sin 2\varphi_{max} + \frac{1}{32}\sin 4\varphi_{max} \right) \right.$$
$$\left. - \frac{L_0}{\delta^4}\left(\frac{5}{8}\sin\varphi_{max} + \frac{5}{48}\sin 3\varphi_{max} + \frac{1}{80}\sin 5\varphi_{max} \right) \right].$$

Finally for an average number N of DNA molecules between two adjacent particles, the total DNA contribution measured is $F_l = N\langle F \rangle_\varphi$. N and φ_{max} being known, this expression is used to fit the contribution of the DNA measured as a function of h the distance between the particles, L_0 and L_p being the only free parameters.

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