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AC electrokinetic manipulation of DNA

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Abstract
The controlled manipulation of molecules is a crucial prerequisite for the emerging field of molecular nanotechnology. AC electrokinetics provide a powerful mechanism for both positioning and inducing conformational changes in molecules. In this paper, we investigate the elongation of fluorescently-labelled DNA strands, which are covalently tethered by one end to gold microelectrodes arranged in an opposing-finger geometry, when exposed to strong ac electric fields. We found that the elongation of the DNA molecules is restricted by the geometry of the gap, and that the observed contour of the elongated DNA molecules coincides with the electric field line pattern. Further, we discuss a potential elongation mechanism and provide evidence that the major contribution to the elongation originates from the ac electrokinetic torque, which is supplemented by a small bias force provided by the electric-field-induced fluid flow.

(Some figures in this article are in colour only in the electronic version)

A key factor for the successful development of molecular nanotechnology will be the availability of a wide range of tools for the manipulation of molecules, molecular complexes, or particles on the molecular scale. The ability to manipulate on the molecular scale is essential to control the position and orientation, and even more importantly the conformation, of molecular complexes, and hence their efficacy. In addition, sophisticated manipulation techniques provide important tools to study fundamental properties of molecular systems. For example, the direct and controlled manipulation of single molecules of DNA has contributed enormously to the understanding of their mechanical properties [1].

A number of techniques are available for controlled manipulation at the molecular scale. Prominent tools in this field are optical [1–3] and magnetic [4] tweezers, where the molecules are labelled with an appropriate bead which is trapped by a laser beam or a magnetic field, respectively. An alternative approach is taken by scanning probe techniques [5], and in particular, atomic force microscopy (AFM) techniques, where the molecules are generally manipulated directly. This has the benefit that the labelling reaction, required for manipulation by tweezers, can be omitted. AFM techniques have been used, for example, to manipulate DNA and to study the force–extension behaviour of DNA [6]. Further, hydrodynamic forces have been employed in manipulation studies, for example, to study the mechanical properties of polymers [7, 8].

Recently, ac electrokinetic manipulation techniques have received considerable attention as an alternative to the techniques mentioned above, and are becoming useful tools in molecular biology and biotechnology. The controlled manipulation of submicron particles by ac electrokinetic techniques has been demonstrated by separating and manipulating cells [9], bacteria [10], viruses [11] and submicron latex spheres [11–13]. In addition, ac electrokinetic manipulation of DNA molecules has led to novel applications such as the concentration of DNA molecules [14, 15], DNA-protein interaction studies [16], and molecular surgery of DNA [17]. However, a detailed understanding of the behaviour of DNA, and in particular of surface-immobilized DNA molecules, when exposed to high frequency ac electric fields, has to be understood in detail to capitalize fully on this technique.

A number of studies have been carried out to investigate the elongation of DNA molecules using ac electrokinetic tools [14,18–20]. Here, we report on a series of experiments dedicated to the investigation of the interaction of the various forces with surface-tethered DNA when the DNA molecules are exposed to strong ac electric fields. We have immobilized four fragments of lambda-DNA of different sizes (15, 25, 35 and 48 kilobasepairs (kb)) onto specific electrodes of an

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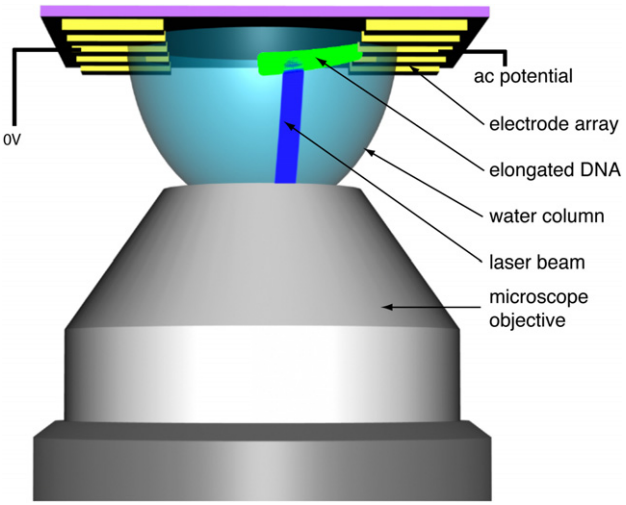

Figure 1. Schematic diagram of the experimental arrangement. Fluorescently-labelled DNA molecules were tethered to the gold electrodes of an electrode array using a thiol linker [22]. The chip-package containing the wafer with the electrode array was mounted upside-down in a custom-built support. A continuous column of water was established between the wafer and the microscope lens. An ac electric potential was then applied to a chosen electrode, while the electrode directly opposite was kept at zero potential.

electrode array comprising two opposing rows of individually addressable electrodes, via a terminal thiol-group. We measured the length of the elongated DNA as a function of electric field, frequency and gap separation, and we compare the elongation pattern of the DNA molecules with the electric field lines obtained from finite element calculations and with previously determined fluid flow patterns [19].

A schematic view of the experimental set-up is given in figure 1. The electrode array comprising individually addressable electrodes, 30 μm wide and 15 μm apart, arranged in two opposing rows, separated by either 20, 30 or 40 μm, were fabricated using standard UV-photolithography. The electrodes were formed by the evaporation of an 80 nm layer of gold on top of a 20 nm adhesion layer of NiCr on Si/SiO_2 wafers using standard optical lithography and lift-off techniques. The wafers were glued and bonded into a chip-package. Prior to attaching DNA to the electrodes, the wafers were cleaned in a 'piranha' solution ($H_2O_2 : H_2SO_4$, 3 : 7 ratio) for 1 h, followed by rinsing in deionized water, ethanol and deionized water again.

The DNA molecules used in all experiments were either lambda-DNA (48 kb, Sigma-Aldrich), or were obtained through appropriate enzymatic restrictions from lambda-DNA (15, 25 and 35 kb). The DNA was fluorescently labelled by diluting it to 50 ng μl^{-1} in TE (10 mM tris-HCl, 1 mM EDTA, pH 8) 1 M NaCl solution and adding the fluorescent intercalator YOYO-1 (excitation/emission wavelength 488/515 nm, Molecular Probes) at an intercalator to basepair ratio of 1 : 8. The contour length of untreated lambda-DNA is 16.5 μm [21], but upon intercalation of the fluorophore YOYO-1 at the concentration used in this work, the DNA molecules lengthen and the contour length increases to approximately 20 μm [2]. Similarly, the contour length of 15 kb DNA increases to 6.5 μm, of 25 kb DNA to 10 μm, and

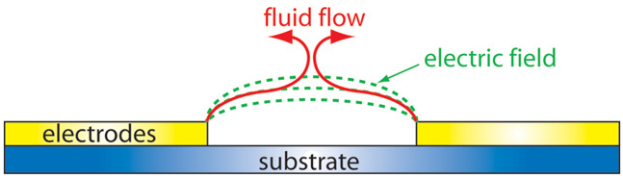

Figure 2. Schematic view of the electric field lines and the fluid flow pattern between two biased microelectrodes. The fluid flow pattern is taken from [19] and the field lines from [20]. Adapted and reprinted with permission from [20] Copyright 2006, American Institute of Physics.

of 35 kb DNA to approximately 15 μm. The DNA molecules were tethered onto the electrodes using a multi-step protocol detailed in [22]. After immobilization, the wafers were kept hydrated in deionized water at all times, and were submerged in deionized water (conductivity $\approx 10^{-5}$ S m^{-1}) for all elongation experiments.

The applied electric fields were generated by applying an ac potential to the particular electrode, while keeping the electrode directly opposite at ground. The electric fields referred to in this work are calculated from the amplitude of the applied potential divided by the width of the gap between the two electrodes. The elongation of the fluorescently labelled DNA molecules when exposed to the ac electric field was investigated with a fluorescence microscope (BX60, Olympus) and a laser-scanning confocal microscope (IX70, Olympus).

To gain a deeper understanding of the underlying mechanism responsible for the elongation of DNA, and in particular the direct influence of the applied electric field, we modelled the electric field around the electrodes using the finite element analysis software, Femlab (Comsol). For the model, the electrodes were represented by a 100 nm-thick layer of gold, separated by gaps of 20, 30 or 40 μm. The suspending medium had a conductivity of 10^{-5} S m^{-1}. Figure 2 shows a schematic side-view of two opposing electrodes separated by 40 μm. The left and right electrodes were set to a potential of 30 V and 0 V, respectively. We note that the electric field is highest directly at the electrode edge, but the variation is small across most of the gap and we can assume that the elongated DNA molecules experience similar field strengths over most of the gap [20].

Figure 3(a) shows the elongation length of DNA of various sizes elongated across a 40 μm wide gap in an applied ac electric field of 375 kV m^{-1} for frequencies between 50 kHz and 1.1 MHz. The elongation is qualitatively similar for all lengths of DNA. Almost no elongation is observed at frequencies below 100 kHz, and upon increasing frequency a sharp increase in the elongation can be measured. The elongation goes through a maximum at around 250 kHz for all DNA fragments, where almost full elongation of the molecules is observed. At higher frequencies, the elongation decreases with increasing frequency, until above 1 MHz almost no elongation can be detected. These findings are in agreement with previous results for larger (500 kV m^{-1}) electric fields on similar systems [19]. Figure 3(b) shows the elongation of 48 kb DNA (20 μm contour length) for different frequencies as a function of the electric field. The behaviour is qualitatively similar for all frequencies, i.e. a steady increase in the elongation is observed with increasing ac electric fields. We

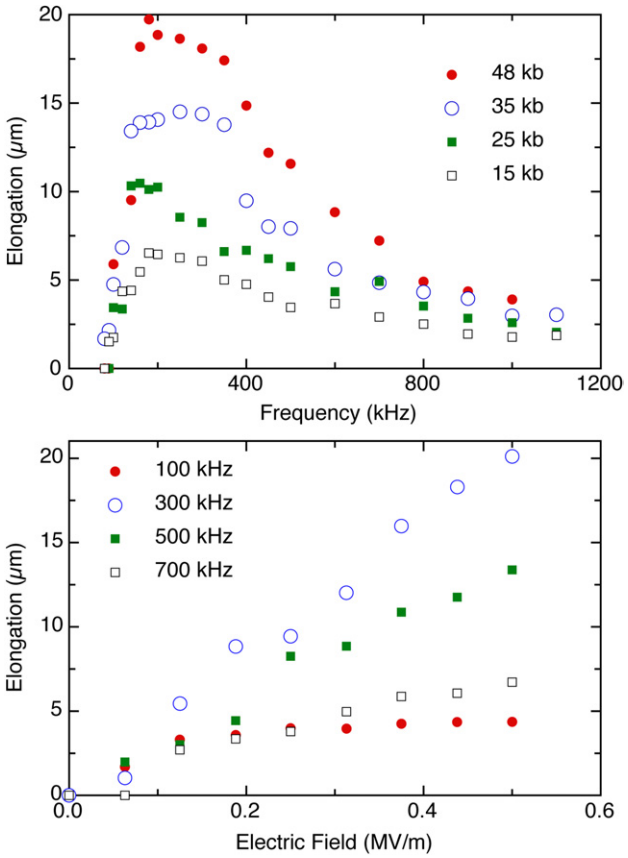

Figure 3. Length of elongated DNA across a 40 μm wide gap as a function of frequency and electric field. Top: DNA of various lengths elongated in a 375 kV m^{-1} electric field. Bottom: 48 kb DNA elongated at various frequencies. The experimental errors are of the order of $\pm$1.5 μm for all experimental configurations used.

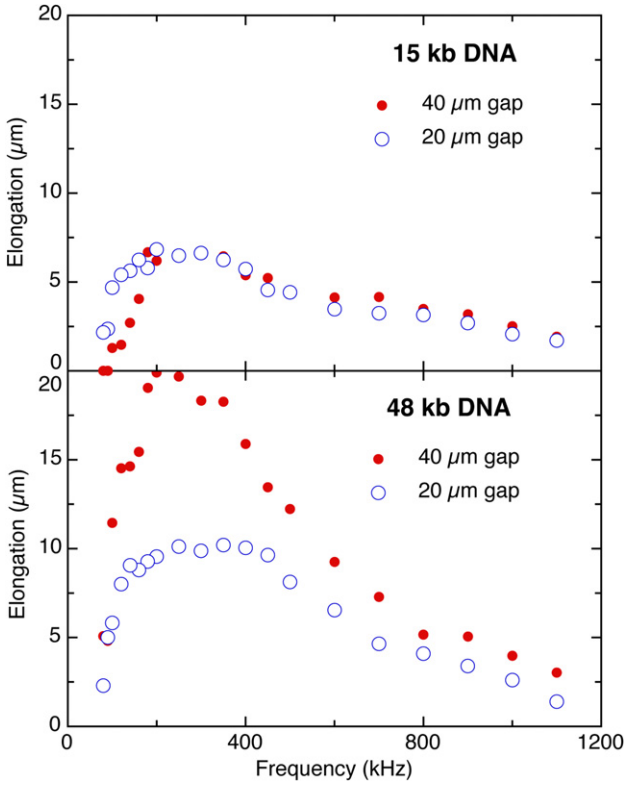

Figure 4. Length of elongated fluorescently-labelled DNA (15 kb (top) and 48 kb (bottom)) across 40 and 20 μm wide gaps versus frequency of the applied electric field.

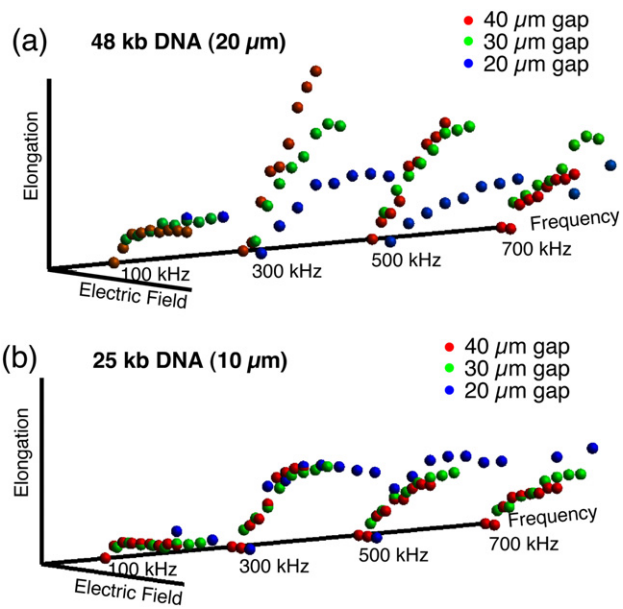

Figure 5. Length of elongated fluorescently-labelled DNA (*a*) 48 kb and (*b*) 25 kb across 40, 30 and 20 μm wide gaps as a function of applied electric field at various frequencies.

note that the maximum elongation, i.e. 20 μm, is obtained at an electric field of 0.5 MV m^{-1} at a frequency of 300 kHz.

In order to investigate the effect of the geometry of the electrodes, and in particular, the effect of the gap-size, we measured the elongation of DNA molecules of various lengths and across different gaps. Figure 4 shows the results for 15 and 48 kb DNA elongated in a 0.5 MV m^{-1} ac electric field across gaps of 40 and 20 μm as a function of frequency. While the elongation of the 15 kb DNA molecules is independent of the gap-size within the experimental uncertainty, the behaviour of the 48 kb DNA molecules is strongly dependent on the gap-size. The 40 μm wide gap allows full elongation and the DNA shows the same qualitative behaviour as at lower electric fields (figure 3(*a*)), but the elongation is substantially restricted in the 20 μm wide gap system. We note that for the smaller gap, the maximum elongation is restricted to about one half of the total gap size, i.e. the DNA molecules cannot be elongated beyond the mid-point of the gap.

Figure 5 shows the elongation of two different DNA fragments, 48 kb and 25 kb DNA with contour-lengths of about 20 μm and 10 μm, respectively, as a function of the applied electric field at different frequencies and across three different gaps. In the case of the 48 kb DNA (figure 5(*a*)) and at a frequency where maximum elongation is expected (300 kHz), a similar effect to that shown in figure 4 is observed—the elongation is restricted to about half the gap size. However, at frequencies where only partial elongation is expected (100 and 700 kHz), the elongation pattern for all gaps is almost identical within experimental uncertainties. For the intermediate case (500 kHz), gaps larger than twice the expected elongation (40 and 30 μm) show very similar behaviour, while the elongation

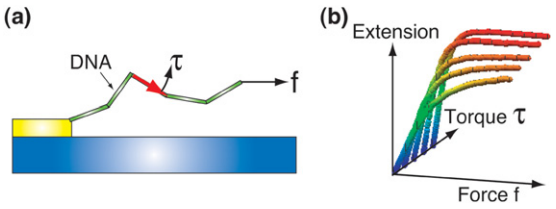

Figure 6. (*a*) Schematic illustration of the elongation mechanism. The DNA molecules are modelled as long strings of connected short segments. In an electric field, a dipole is induced in each segment, and the resulting electronkinetic torque τ aligns the segments with the electric field. However, in order to achieve elongation, an additional bias force, f, i.e. a point force pulling at the free end of the DNA, is required so that a forward, parallel alignment is favoured over an antiparallel alignment. (*b*) Resulting elongation as a function of bias force, f, and torque, τ (data taken from [25]). Reprinted with permission from [28] Copyright 2006, Imperial College Press, London, UK.

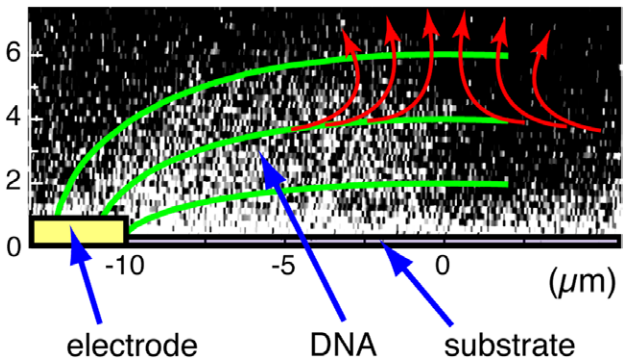

Figure 7. Side view of elongated, fluorescently-labelled DNA in an elongation-restricted system (48 kb DNA across a 20 μm gap). The lines indicate the electric field-lines and the arrows the induced fluid-flow. Adapted and reprinted with permission from [20] Copyright 2006, American Institute of Physics.

across the small gap (20 μm) is substantially restricted. In the case of the 25 kb DNA (figure 5(*b*)), where the contour length of the molecules does not exceed half the gap size of even the smallest gap, the elongation patterns of the three different gaps coincide at all frequencies and electric fields within experimental errors.

Charged polymeric molecules under the influence of external forces, for example DNA exposed to an ac electric field, can be described by the wormlike chain (WLC) model [23], a continuous limit ($l \to 0$) of the Kratky–Porod (KP) model [24], which describes the polymeric molecules as a succession of N segments of length l. DNA is highly polarizable, and when exposed to an electric field, a dipole is induced along the backbone of the DNA. As a result, the electric field exerts a torque τ on the DNA through interaction with the induced dipole, which causes the individual DNA segments to align with the electric field. The alignment of the DNA segments is either parallel or anti-parallel with the electric field, as both orientations are energetically equal. Since both orientations are equally probable, no elongation results, and only when an additional bias force, f, for example, a point force pulling on the free end of the DNA, is applied, the forward, parallel alignment of the DNA segments with the electric field is favoured over an anti-parallel alignment, resulting in elongation.

The elongation of a polymeric molecule as a result of an external applied electric field supplemented with a small bias force has been calculated [25] and the results are shown in figure 6. It can be seen that elongation only occurs if the torque τ is supplemented by a bias force, f. In an inhomogeneous ac electric field such as is present in our experimental set-up, a dielectrophoretic force acts on the induced dipoles [26]. However, in the present case, the dielectrophoretic force points towards the ends of the molecules that are tethered to the surface and therefore a dielectrophoretic bias force would not lead to elongation. However, the strong ac electric fields lead to fluid flow in the solvent surrounding the DNA, which in turn leads to movement of the molecules [7, 12, 19, 27]. We have investigated the fluid flow that occurs in our system and a schematic illustration is given in figure 2. We found that the fluid flow follows the electric field lines and points in the direction suitable to lead to elongation anywhere in the gap apart from the centre, where it points upwards, perpendicular to the electric field lines. The strength of the fluid flow decreases with increasing ac electric field frequency but was qualitatively similar for all conditions used in our experiments [19]. Below, we show that the major contribution to the elongation of the DNA molecules in our system results from the ac electrokinetic torque τ which acts on the DNA molecules, supplemented by a bias force, f, provided by the electric-field-induced fluid flow.

Above about 300 kHz, the decrease in elongation coincides with the decrease in fluid flow for increasing frequencies. However, the reduced elongation is also a direct result of the decrease in torque, owing to the increasing lag between the ac electric field and the induced dipole [19]. On the other hand, below 200 kHz, the fluid flow increases with decreasing frequency, whereas the elongation decreases sharply. This indicates that the fluid flow plays only a limited role in the elongation mechanism, i.e. it provides only a supplementing bias force and not the major contribution to the elongation. By inspecting the fluid flow in the centre of the gap (figure 2) and by taking into account the effect of the fluid flow on the elongation (figure 6), it becomes obvious that the elongation is only possible up to the mid-point of the gap. At the mid-point, the fluid-flow changes direction and points upwards away from the electrodes, i.e. it is perpendicular to the electric field lines and does not provide the required bias-force anymore.

However, the results discussed above were obtained by top-view, two-dimensional imaging which does not allow one to investigate fully the elongation of DNA in the centre of the gap in an elongation-restricted system. This is of particular importance, as any other contribution of the fluid-flow, other than providing a supplementing bias-force, would lead to an upturn of the DNA in the centre of the gap. Figure 7 shows the side-view of 48 kb fluorescently labelled DNA (contour length approximately 20 μm) elongated at 210 kHz and 0.6 MV m^{-1} across a 20 μm gap. The electric field lines are superimposed onto the image. The arrows indicate the fluid-flow in the centre of the gap. Although the elongation is restricted to about one half of the contour length of the DNA, the DNA only extends to the centre of the gap. Further, no indication of an upturn is observed, in agreement with the model of elongation discussed here. We note that the contour of the area containing

the elongated DNA closely resembles the shape of the electric field lines.

In conclusion, we have investigated the elongation of DNA molecules when exposed to an ac electric field for a number of different frequencies, electric fields, DNA lengths and across different gap separations. We found that the elongation is restricted by the geometry of the electrode arrangement, i.e. elongation is only possible up to the centre of the gap. A possible mechanism for the elongation was discussed and we presented evidence that the main contribution to the elongation stems from the electrokinetic torque which is supplemented by a bias force provided by the component of the electric-field-induced fluid flow parallel to the electric field lines. We demonstrated that the contour of the area containing the elongated DNA resembles the shape of the electric field lines, even for gaps smaller than twice the contour length of the DNA molecules, where the molecules only extend to the centre of the gap. No upturn in the centre of the gap was found although the major component of the fluid flow is vertical at this position.

Acknowledgments

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