Single molecule study of DNA conductivity in aqueous environment

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The dc electrical conductivity of double stranded DNA is investigated experimentally. Single DNA molecules are manipulated with subpiconewton force and deposited on gold nanoelectrodes by optical traps. The DNA is modified at its ends for specific bead attachments and along the chain to favor charge transfer between the DNA base pair stack and the electrodes. For an electrode separation of 70 nm we find, in aqueous environment, electrical resistances above 100 GΩ indicating that even for weak stretching the double helix is almost insulating at this length scale.

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The question of electrical conduction through DNA molecules has recently attracted a lot of interest in biology as well as in the field of molecular electronics. On the one hand, charge transfer along the double helix chain is involved in biological processes like oxidative damage or repairing of DNA. On the other hand, DNA also appears as an attractive candidate for molecular wiring, because sophisticated DNA networks can be realized in a bottom-up approach, using self assembling by complementary base pairing together with the molecular biology toolkit for specific DNA preparation and modification.

Several decades ago, it was suggested that double stranded DNA could mediate charge transfer due to hybridization of orbitals between the stacked bases pairs [1]. While biochemical and electrochemical studies have shown that charge transfer occurs on short ranges of a few tens of nanometers [2], the long range intrinsic conductivity of DNA still is a controversial subject. Published experimental results range from insulating to conducting behavior [3]. Such discrepancies may, at least partly, be due to the difficulty of establishing a contact between the molecule and the electrodes and of controlling the stretching state of the DNA molecules. Stretching increases the base to base distance and is thus expected to influence strongly the charge transfer rates. We believe that the local environment of the DNA also plays an important role. A recent scanning tunneling microscopy (STM) measurement in liquid shows that oligonucleotides with periodic sequences are moderatly conducting with resistances in the range of 10 MΩ for the poly GC sequence [4]. In dry or vacuum environments, two major trends can be distinguished. Most of the electrical investigations have been carried out on DNA molecules adsorbed on substrates (mica typically), and DNA is most often found to be insulating, even in contactless experiments [5]. Such adsorbed molecules result from molecular combing by a receding meniscus which exerts a high force leading to a significant overstretching of the molecule [6], which could drastically reduce the electronic overlap between the stacked bases. Some investigations have been done on DNA bundles that are not adsorbed on the substrate but suspended between electrodes; semiconducting behavior is then observed [7]. Such differences could reflect the fact that a too strong interaction of DNA with a substrate and/or a too strong stretching of the molecule could inhibit its intrinsic conductivity, as suggested by different groups [8–10].

Here we use optical trapping, a single molecule manipulation technique which has been widely used to investigate the elastic properties of DNA [11], to extend single DNA molecules with a low calibrated force, and to deposit them on gold nanoelectrodes for subsequent conductivity measurements. By modifying the stretching force one can induce a significant variation in the base to base distance and, hence, in the electronic overlap between the stacked bases. In the short range tunneling mechanism, the charge transfer rate should decrease exponentially with the base to base distance. Moreover, the applied force can also influence the conformational dynamics that is supposed to be responsible for long range charge transfer in aqueous solutions [12].

As illustrated in Fig. 1, a double trap allows independent manipulation of two beads attached to the opposite extremities of a single DNA molecule and precise alignment and positioning across the electrodes. The same configuration also allows for single molecule conductivity measurement in four point geometry, eliminating the contribution of the contact resistances. As the measurements are done in a liquid environment, there is no drying step and therefore the stretching imposed to the molecule is controlled and weak.

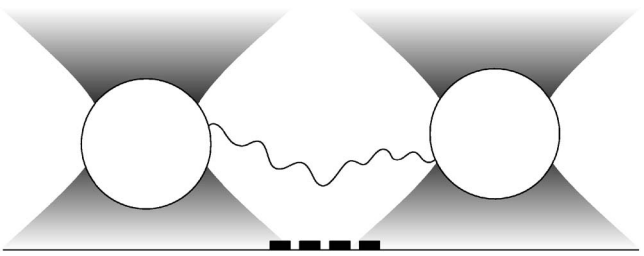

FIG. 1. Principle of the experiment. A single DNA molecule is precisely positioned by optical trap micromanipulation on electrodes under liquid, thus allowing for direct electrical measurements. Disulfide groups are introduced along the chain to enhance the electronic coupling to the electrodes.

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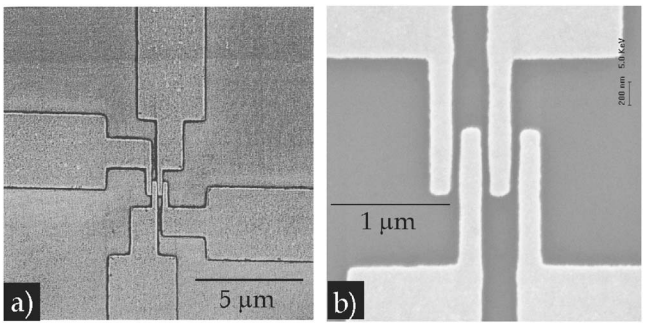

FIG. 2. Scanning electron microscopy (SEM) images of the gold nanoelectrodes on oxidized silicon. The bars indicate the scales. The 160 nm wide electrodes are separated by 70 nm gaps (see Ref. [13]).

As the SiO_2 surface charge and the intrinsic charge of the DNA molecule are of the same (negative) sign, the DNA joining the electrodes is subject to an electrostatic repulsion which preserves strong adsorption to this surface. The double helix should therefore conserve its physiological B-helix conformation.

The nanoelectrode samples used in this work each consist of four gold fingers [length 1 μm, height 40 nm (including the 8 nm attachment layer), width 160 nm], separated by 70 nm. The samples have been fabricated by metal deposition and electron beam lithography on silicon wafers covered by a 100 nm SiO_2 oxide [13]. As shown in Fig. 2, the arrangement of the four electrodes allows for four-contact measurements. In order to manipulate DNA molecules above such an opaque and microstructured surface, we developed a double optical trap based on an upright microscope with a fixed stage and a movable long working distance water immersion objective allowing easy access to the electrodes. The same objective is used for both trapping and imaging of beads in an open fluid cell. Polarizing cubes permit one to form two optical traps. The position of the first trap is controlled by a piezomirror to adjust the lateral separation between the traps from 0 to 10 μm. The laser beam defining the second trap is imaged on a position sensitive detector to measure the bead displacement in the trap which is proportional to the stretching force applied to the molecule linking the two beads. Trap calibration is performed by power spectrum analysis of the Brownian motion of a trapped bead. A more detailed description of this setup can be found elsewhere [14].

Thiol functionalization of the ends of small organic molecules is an efficient way to form strong chemical bonds as well as good electrical contacts between the molecules and gold surfaces [4,16]. In our experimental configuration with a long DNA molecule we need to establish the electrical contacts along the double strand. Therefore sulphur containing groups (thiol or disulfide groups) must be introduced along the molecule. We prepared two types of ten kilobase double stranded DNA (contour length $L=3.3$ μm) by polymerase chain reaction (PCR). The unmodified DNA has at its ends digoxygenin and biotin groups introduced by 5′ labeled PCR primers. In addition to these terminal functionalizations the modified DNA possesses a random lateral amine functionalization obtained by the incorporation of amine modified nucleotides during the enzymatic amplification [17]. After coupling with a NHS-ester crosslinker which possesses disulfide groups and reacts with the amines, the DNA becomes laterally functionalized with disulfide groups [18].

Two different tests have been carried out to verify that the disulfide modified DNA sticks specifically on gold surfaces. For both tests, the protocol was rigorously the same for the modified DNA and the unmodified one (coupling with the crosslinker, purification steps). The first test uses colorimetric detection based on distance-dependent optical properties of gold nanoparticles [19]. After mixing gold nanoparticles and DNA, the color changes from pink to light purple with modified DNA, evidencing gold nanoparticles bridging through disulfide modified DNA, while the color remains pink for unmodified DNA.

As a second test, we checked the adhesion on gold by fluorescence microscopy at the single molecule level. A PB buffer containing the DNA molecules tagged with YOYO-1 fluorophores is introduced by capillary forces between a gold surface and a cover glass. With unmodified DNA, we observe that most of the molecules are aligned in the flow direction, the remaining ones appearing as dots. In this case the molecules stick to the gold surface by one extremity, either through the digoxigenin protein or because the attachment of the DNA's extremities is easier than that of its inner parts [20]. The molecules are then stretched by the flow and then stick to the surface by their second end. DNA molecules reaching the gold surface after the flow stops maintain their random coil shape and appear as dots. With the modified DNA, most of the molecules appear as dots. We attribute the very poor stretching efficiency in this case to the specific interaction between the disulfide groups and the Au surface. The molecule sticks entirely as a random coil before being significantly stretched.

The overall conclusion of these tests is that amine modified nucleotides are readily introduced within the DNA sequence during PCR, and the subsequent coupling efficiently introduces along the chain disulfide groups which are accessible for coupling to gold surfaces.

The DNA molecules (concentration 1 ng/μl) are incubated for 3 h with 1 μm diameter silica beads coated with streptavidin or antidigoxygenin. Then 4 μl are injected in a 200 μL volume of PB buffer (10 mM Na_2HPO_4, KH_2PO_4) previously deposited on the gold electrodes. The sample is observed under microscope and a pair of beads with correlated movements is selected. Each bead is trapped in a different trap. The presence of the molecule linking the beads is checked by measuring the force/extension curve of the molecule [14], as the trap to trap distance is increased. A typical curve is shown in Fig. 3. By translating the sample holder horizontally the molecule is positioned above the electrodes, which appears out of focus in Fig. 4(a). The objective is moved down until the beads touch the surface as shown in Fig. 4(b). The traps are then turned off. Typically this deposition process is performed with an extension x of 2 μm. This corresponds to a relative DNA extension of $x/L=0.6$ and a very low force of about 0.1 pN, i.e., the entropic regime of polymer elasticity.

At a first glance a difficulty appears, namely in our ex-

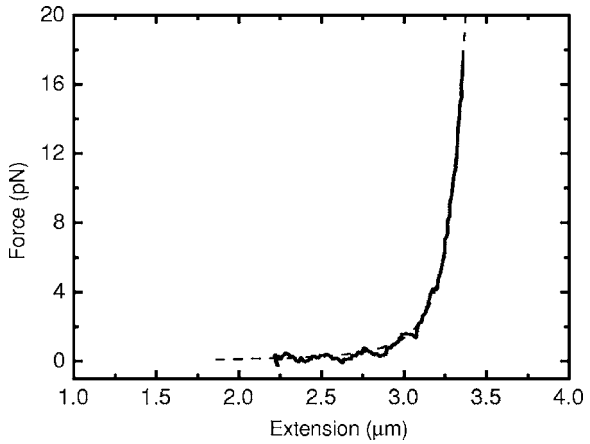

FIG. 3. Force vs extension curve of a single DNA molecule in aqueous environment, measured with the double optical trap. The dashed line shows a calculation based on a modified Marko-Siggia wormlike chain model (see Ref. [15]), using the following fit parameters: a contour length of 3.5 μm, a persistance length of 50 nm, and an elastic modulus of 1000 pN.

perimental geometry the probability for a DNA molecule to encounter the electrode surface may be low since the radius of the beads ($R=0.5$ μm) strongly exceeds the electrode height ($h=40$ nm). However, as shown by the following theoretical consideration, the room temperature thermal motion of the DNA is expected to be sufficient to assure that the central part of the extended molecule touches the surface on a typical time scale of a few seconds, while the delay between the deposition and the conductivity measurement is about 10 min.

We consider the case where the DNA molecule joins the two beads at equatorial positions. This is the typical geometry obtained after DNA trapping and extension, provided the beads are sufficiently uniform in shape and scattering properties that the small torque ($\sim$0.1 pN 0.5 μm=50 pN nm), that develops when a molecule attached to a nonequatorial point on a bead is extended, suffices to induce rotation in the trap. If such rotation does not occur the situation is more favorable for DNA electrode encounter to occur since then at least one of the attachment points often is close to the surface. Recently optical trapping of spherical beads was used to form a plectonemic superhelix by DNA braiding at linear forces of up to 30 pN [21]. With a compaction of at least 35 nm for each additional turn, this corresponds to a torque above 30 pN 35 nm $\cong$ 1000 pN nm, which suggests that also in our case the beads do not rotate in the optical trap.

For the characteristic time τ of our thermally activated process we consider the relation

$$\tau^{-1} = \nu \exp\left(-\frac{\Delta G}{k_B T}\right), \tag{1}$$

with an energy barrier of height ΔG and an attempt frequency ν. To touch the surface the DNA has to be extended from the initial length L_0 to $L=\sqrt{L_0^2+4R^2}$. For $L_0=2$ μm, $R=0.5$ μm, $F=0.1$ pN, we get an extension of 240 nm and a barrier height of $\Delta G \simeq F\,(L-L_0)=6k_BT$. To estimate the attempt frequency ν, we use a hydrodynamical description of the transverse excursion $z(t)$ of the DNA,

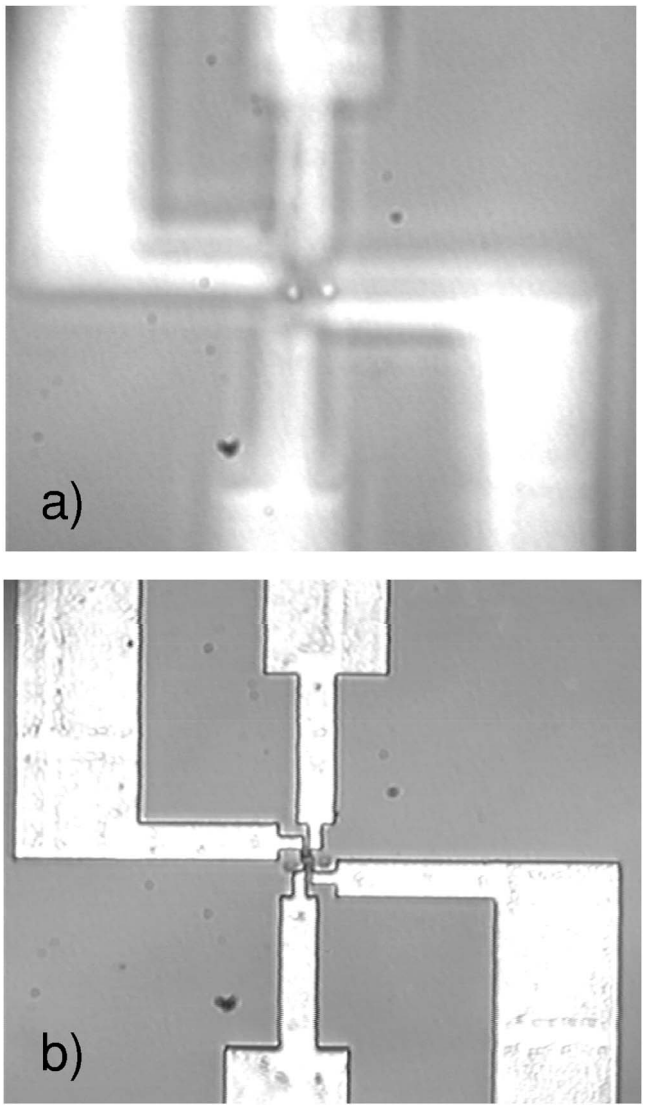

FIG. 4. Optical microscope images of the deposition of a molecule linking two beads on the electrodes. The beads are (a) laterally positioned above the electrodes which appear unfocused, then (b) down translated to the surface. Because the beads are trapped and imaged by the same objective, they are always in focus.

$$\xi \frac{\partial z}{\partial t} = F_z \simeq F \frac{2R}{L}, \tag{2}$$

where ξ is an effective friction coefficient for transverse motion of the DNA molecule and F_z is the transverse component of the fluctuating stretching force F. The friction coefficient of a rigid rod (length L, diameter b) for motion perpendicular to its axis, given by $\xi_\perp = 4\pi\eta L/\ln(L/b)$, can be used to estimate the ξ value for the transverse DNA motion. For our 10 kb DNA we thus get $\xi = 1.85\times10^{-9}$ N s/m, a value close to the result of 1.75×10^{-9} that is obtained when the value of 7×10^{-9} measured by Meiners and Quake for λ DNA single molecules (48 502 bp) is rescaled to our

DNA length [22]. We evaluate Eq. (2) for an amplitude $\Delta z = R$ and find

$$\nu^{-1} = \Delta t = \frac{\xi L}{2F} \simeq 0.02 \text{ s}. \quad (3)$$

From Eq. (1) we finally obtain the characteristic time $\tau = 7.5$ s. This result indicates that the extended molecule touches the surface on a time scale (seconds) that is short compared to the delay between the deposition and the conductivity measurement (about 10 min) and therefore suggests that the reaction between the deposited DNA molecule and the gold electrode can occur as anticipated. The presented model is appropriate to estimate the DNA motion for the experimentally used forces of 0.1 pN or above, but should not be used in the zero force limit where the force is too small to significantly perturb the molecule (in our case a few femtonewtons or below).

We limit the voltage applied between two electrodes to low values (60 mV rms) in order to avoid redox reactions at the electrode/electrolyte interfaces which would otherwise give rise to significant parasitic current and could ultimately even destroy the electrodes. To circumvent the ionic conductivity of the electrolyte itself, we work at very low frequencies using numeric lock-in techniques. In this case, the electric field in the volume of the liquid is completely screened by the double layers close to the metallic surfaces. In other words, the capacitive impedance due to the double layers on the electrodes is much larger than the electrolyte bulk resistance [23].

Prior to the introduction of DNA in the buffer, we checked that below 100 Hz the device is essentially capacitive (capacitance C about 0.6 nF) and that the current modulus increases linearly with frequency f in the range 0.01–100 Hz. We choose to work at $f = 0.01$ Hz to reduce this capacitive current as much as possible (in the 10 pA range) to get a better sensitivity. At these long time scales (lock-in integration constant of 300 s), a small drift of the device is observed. This drift being more important after the cleaning step [24], the device is incubated overnight under buffer to stabilize the electrochemical state of the surface.

The electrical measurements are first performed in the PB buffer containing the molecular construction but prior to the molecule deposition. The beads concentration is low enough that a spontaneous deposition on the electrodes is unlikely. Then a molecule is brought from the volume of the buffer and deposited on the electrodes as explained above. The electrical measurements follow immediately the deposition process with unchanged experimental conditions (temperature, pH, ionic strength). As divalent magnesium is thought to play a major role in the conduction of wet DNA [3], we have performed these measurements either in a PB buffer or in a PB buffer containing 1 mM of magnesium. We measured about 50 current-voltage characteristics. Typical characteristics are shown in Fig. 5, with and without magnesium, both preceeding or following the molecule deposition. Within the experimental error, the current modulus is the same with and without the deposited DNA molecule and the current remains 90 deg out of phase with the voltage. Therefore, even in the presence of magnesium, the deposition of the DNA molecule does not introduce any significant contribution to the conductance of the device. We have also done these measurements on unmodified DNA and find the same result.

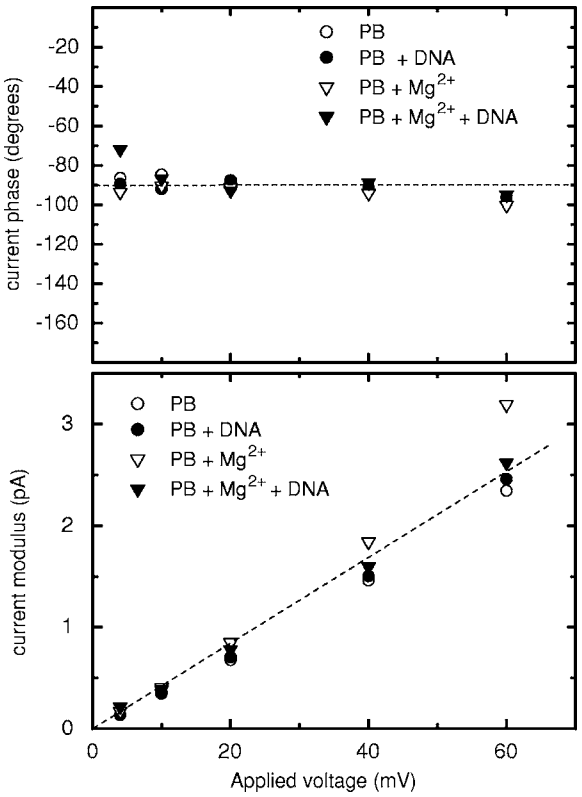

FIG. 5. Two-contact $I(V)$ characteristics measured at 0.01 Hz in aqueous environment for the sample before and after deposition of a DNA molecule. The buffer is either PB 1× or PB 1× with 1 mM $MgCl_2$. Current modulus (bottom) and current phase (top) are presented.

As any small contribution of DNA in current should appear in phase with the applied voltage, i.e., 90 deg out of phase with the main capacitive current of the device, the most sensitive experimental parameter is the phase. In Fig. 5 the phase remains equal to 90 deg within error bars of about ±5 deg. Therefore any variation of the phase larger than 5 deg should be clearly resolved experimentally. For our experimental parameters this corresponds to $[2\pi fC \tan(5^0)]^{-1} \cong 300$ GΩ. We thus conclude that the dc-resistance of the 70 nm long DNA double strand is higher than 100 GΩ in liquid [25]. We applied different stretching forces between 0.1 and 1 pN, but always observed such high resistance. Since the electrical response is governed by the liquid and not by the DNA molecule, the four probe configuration does not provide an advantage in this high resistance regime. Therefore two adjacent probes have been used in the measurements.

It is interesting to compare our results with those obtained in another configuration where the influence of the surface is reduced, i.e., free-hanging DNA in air or in vacuum. For 10 nm long poly(G)-poly(C) DNA oligomers [26] a resistance above 1 TΩ was measured at moderate bias, while the current rises rapidly above a threshold of a few volts, indi-

cating a large band gap semiconductor behavior. This high voltage range cannot be investigated under liquid, since electrochemical reactions at the electrodes must be avoided. Conductance measurements performed in aqueous solution on double stranded DNA oligomers of less than 5 nm in length [4] have shown a resistance that increases linearly with length for periodic $(GC)_n$ sequences (30 MΩ for $n=7$), and a sharp increase of resistance for more complex $GCGC(AT)_mGCGC$ sequences with an exponential dependance on m (3 GΩ for $m=2$). Our observation that $R>100$ GΩ for a 70 nm long random sequence is in agreement with these results.

In conclusion, we have performed electrical measurements on single DNA molecules in mild environmental conditions. The molecule is maintained in aqueous environment to preserve its helix B conformation. The molecule has been gently elongated for deposition across the electrodes, but significant stretching which could affect the base stacking and therefore the charge transfer through the coupling of the bases-bonds is avoided. A statistical lateral functionalization of the molecule has been done to optimize the electrical contacts between the DNA molecule and the gold electrodes. Under these experimental conditions, the molecule appears to be insulating in aqueous environment ($R>100$ GΩ for a 70 nm distance).

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[17] The 10 kb DNA fragment is part of the λ phage. The protocol available for the "expand long template PCR system" (Roche cat. N1681834) has been adapted to allow for the statistical incorporation of a modified nucleotide that possesses an amine group 5.(3.aminoallyl)dUTP (AAdUTP, Sigma) instead of the unmodified dTTP. We choose for the modified DNA a AAdUTP:dTTP concentration ratio of 0.1:1, a value for which the PCR yield is almost the same as that of the standard PCR for unmodified DNA (0:1 ratio). If the incorporation rate were the same for dTTP and AAdUTP, we expect to have about 25 amine modified base on a 160 nm modified dsDNA fragment (corresponding to the width of a nanoelectrode). The amine incorporation in modified DNA has been checked by fluorescence by coupling to a monofunctional NHS-ester Cy5 dye (Amersham), taking unmodified DNA as a reference.
[18] 1 μL of fresh solution of dithiobis(sulfosuccinimidyl-propionate) (DTSSP, Pierce) of concentration 1 mM is added to 500 μL of the DNA sample of concentration 0.1 nM in PB 1× buffer. It is incubated for 30 min then quenched with tris buffer and thoroughly purified by filtration.
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[23] In the ionic strength ~10 mM, the PB 1× (or PB 1×+1 mM $MgCl_2$) electrolyte has a typical resistivity $\rho\sim10^3$ Ω cm and a Debye screening length $\lambda_D\sim3$ nm. With electrodes of surface S and separation $e=70$ nm, the bulk resistance of the liquid $R=\rho e/S$ and the double layer capacitance at one electrode $C=\epsilon_0\epsilon_r S/\lambda_D$ are related by the relation $RC=\rho\epsilon_0\epsilon_r e/\lambda_D\sim10^{-7}$ s. Therefore we expect $R<1/(2\pi fC)$ for frequencies below 1 MHz. Strictly speaking, one should take into account the influence of the parts of the connecting lines that are in liquid; as these large surfaces are far from each other they contribute only slightly to a decrease of the bulk resistance R but they increase strongly the capacitance C of the system. Therefore, the cutoff frequency is reduced; we find experimentally that for frequencies below 100 Hz the device has a pure capacitive response.

[24] The cleaning procedure consisted of the deposition of a drop of sulfochromic acid (1 min) followed by rinsing with water.

[25] We also performed electrical measurements in dry conditions. In this case also, the device remains essentially capacitive (in this case the much smaller capacitance, about 20 pF comes from the coupling through the silicon substrate). After the molecule deposition followed by a complete removal of the liquid buffer, the $I(V)$ characteristic is measured and compared to the values obtained prior to the molecule deposition and in the same dry conditions. Here again no noticeable change is obtained. We find that the resistance of the 70 nm long DNA double strand is higher than 10 TΩ in dry conditions.

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