

Insulating Behavior of λ -DNA on the Micron Scale

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We have investigated the electrical conductivity of λ -DNA using DNA covalently bonded to Au electrodes. Thiol-modified dTTP was incorporated into the “sticky” ends of bacteriophage λ -DNA using DNA polymerase. Two-probe measurements on such molecules provide a hard lower bound for the resistivity $\rho > 10^6 \Omega \text{ cm}$ at bias potentials up to 20 V, in conflict with recent claims of moderate to high conductivity. By direct imaging, we show that the molecules are present after the measurements. We stress the importance of eliminating salt residues in these measurements.

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The question of whether DNA is electrically conducting has generated broad interest. The initial spurt of interest arose in photoexcitation experiments which were interpreted in terms of long-range electron transfer [1]. In the past few years, there have been upwards of 20 papers reporting the results of more direct electrical measurements ranging from contactless measurements at microwave frequencies to dc measurements. A distressingly wide range of conductivity values—from $\rho < 10^{-4} \Omega \text{ cm}$ to $\rho > 10^6 \Omega \text{ cm}$ —has been reported [2–6]. Proximity-induced superconductivity in DNA has also been claimed [7]. Recently, local polarization measurements by “electrostatic force microscopy” have been used to show that λ -DNA is insulating [8,9]. We note, however, that the force-microscopy experiments probe conductivity at relatively weak bias potentials.

In many of the dc measurements, contact with the metal electrodes (usually Au) was achieved by laying down the molecules directly on the electrodes. Although expedient, this approach raises several concerns. It is very difficult to prove that the DNA molecule is in direct physical contact with the electrodes. Even if contact is attained, the weak physical adhesion between DNA and Au may produce an insulating contact and possibly account for the wide variation in reported resistivities [10]. A recent experiment on octanedithiol [11] has shown that deliberate chemical bonding between organic molecules and metal electrodes is a prerequisite for achieving reproducible conductivity results. Thus, a better approach would be to achieve direct chemical bonding between the open ends of λ -DNA and Au. The bonds should be strong enough to withstand shear forces in a flow and should survive the measurement process. A second concern is the shunting effect of buffer residue. Because of its finite conductance, the buffer salts which coat the electrodes and substrate produce a spurious conductance signal. Hence, adequate salt removal is important. We report the results of experiments performed along these lines. Our results show that λ -DNA is a good insulator up to bias potentials of 20 V.

Chemical binding between organic molecules and Au is usually achieved by the Au-thiol (SH) chemical bond [12]. Commercially available oligonucleotides modified to incorporate the thiol group usually have carbon-chain spacers (C3 or C6) between the thiol group and DNA [3,6,13], which may present barriers to electron transfer. To avoid the spacer problem, we adopted an approach in which the DNA base pair is bound *directly* to gold electrodes by a Au-thiol bond. This approach should provide the most direct conductance channel between the gold electrode and the putative electronic “ π -way” proposed for the DNA helix [14].

λ -DNA is a double-stranded DNA helix comprised of 48 502 base pairs (length $\sim 16 \mu\text{m}$). At the extremities, there are single-stranded 12-base 5' overhangs (“sticky ends”), with the complementary sequences

$$5' - GGG CGG CGA CCT,$$

$$5' - AGG TCG CCG CCC,$$

where *A*, *C*, *G*, and *T* are the nucleotides adenine, cytosine, guanine, and thymine, respectively. Our technique relies on the incorporation of *T*'s modified to include the desired thiol group [15,16]. The sticky ends are filled in by a standard reaction [17] using the Klenow fragment of DNA polymerase and the three deoxynucleoside triphosphates dATP, dGTP, and S^4 -dTTP [see Fig. 1(a)]. Because of the preponderance of modified dTTPs (and absence of dCTP) in solution, we can incorporate a significant number of modified *T*'s at both ends of each DNA molecule [19]. To prevent the Klenow fragment from excising *T*'s that are not Watson-Crick matched to the template, we use a mutated form of the Klenow fragment which lacks the 3' \rightarrow 5' proofreading activity [20].

We tested the incorporation of the nucleotides into the DNA ends by a ligation assay [21]. Unmodified λ -DNA is readily ligated by T4 DNA ligase to form multimers. In the modified λ -DNA, however, the sticky ends—now filled in by the incorporated bases—are blunt, and multimer formation is strongly suppressed. The reaction products were analyzed by pulsed-field gel electrophoresis. As

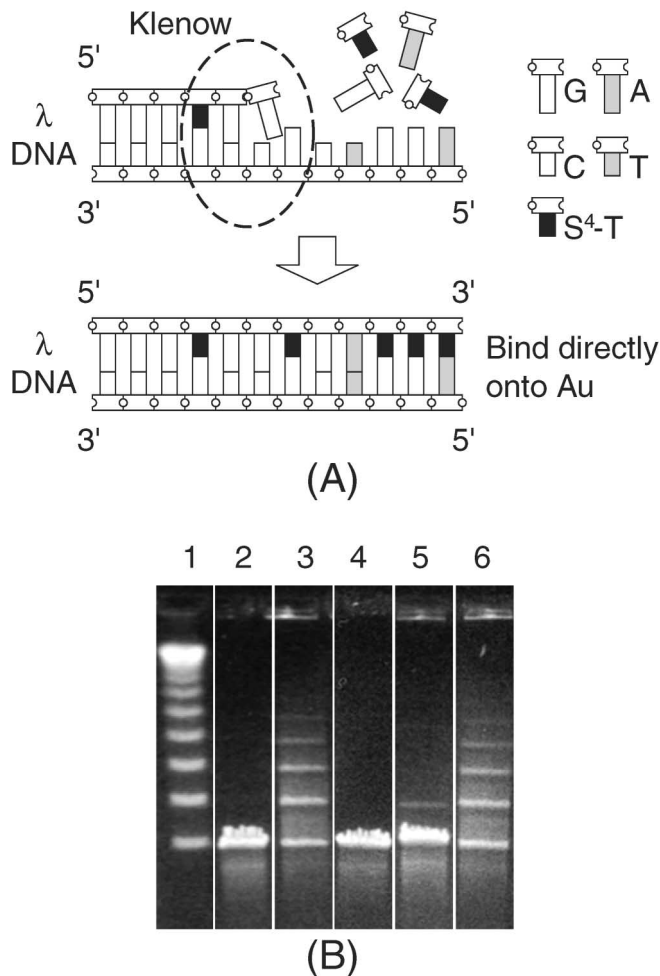


FIG. 1. (A) The schematic of the incorporation of deoxynucleoside triphosphates into λ -DNA sticky ends using the Klenow fragment ($3' \rightarrow 5'$ exo^-); (B) pulsed-field gel (PFG) electrophoresis of thiol-modified and natural λ -DNA before and after ligation [18]. Lane 1: λ -DNA PFG marker (New England Biolabs); 2: unmodified natural- λ monomers; 3: ligated natural- λ multimers; 4: thiol-modified λ -DNA (HS- λ) monomers; 5: HS- λ after ligation, with no significant multimer formation; 6: control- λ after ligation, proving that the presence of unincorporated S^4 -dTTP and other dNTPs does not inhibit the ligation reaction.

shown in Fig. 1(b), unmodified λ -DNA (“natural- λ ”) was efficiently ligated (lane 3). To control for the possibility that unincorporated S^4 -dTTP inhibited ligation, λ -DNA monomers were ligated in the presence of all three dNTPs minus the polymerase (“control- λ ”), and the ligation was also efficient (lane 6). The majority of the thiol-modified λ -DNA (“HS- λ ”), however, remained as monomers (lane 5). This provides strong evidence that the protocol is effective in incorporating bases into the ends of λ -DNA.

Using standard photolithography, we constructed Au electrodes on a quartz substrate in parallel strips, $4 \mu\text{m}$ wide and 5 mm long, and separated by 4 or $8 \mu\text{m}$. The Au surfaces were rigorously cleaned [22] before depositing

the modified DNA. At several stages during these experiments, it was important to observe the molecules in an optical microscope. To image the thiol-modified DNA molecules, we stained them with the fluorescent intercalating dye TOTO1 (Molecular Probes), and then loaded them on the chip. After a 20-min incubation period, many of the molecules were observed to be attached to the electrodes at one end. The unattached molecules were carefully rinsed in $1 \times \text{TE}$ [23]. The chip was then covered with a clean coverslip, and a flow of the buffer solution was applied perpendicular to the electrodes. We observed that DNA molecules anchored at one end were stretched by the buffer flow to bridge the space between the electrodes. Many of these molecules subsequently attached to the second electrode by their free end. After this occurs, the flow may be repeatedly reversed to demonstrate that the anchored DNA molecules bow out with the flow while their ends remain anchored (panels A and B of Fig. 2; see video in Ref. [24]). This is direct evidence that chemical binding between the ends to Au is much stronger than physical adhesion of the rest of the molecule to either quartz or Au. (For the specific DNA samples used in the resistivity measurements, we carried out the

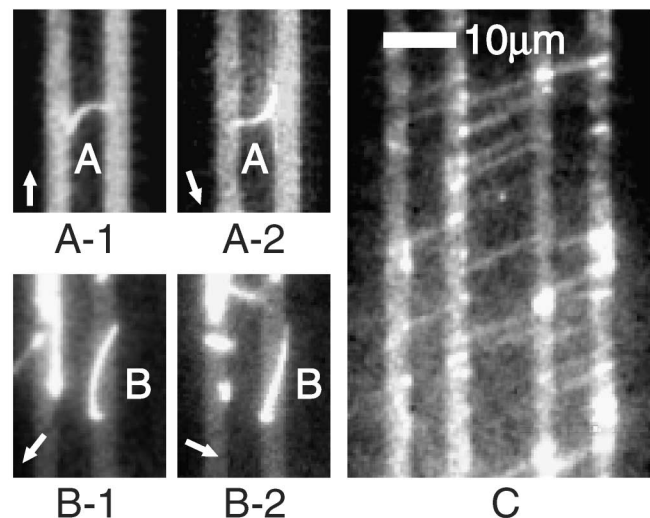


FIG. 2. Images of HS- λ DNA on quartz chips with Au electrodes. The DNA molecules were prestained with TOTO1. Observations were through a Nikon Eclipse TE300 inverted microscope equipped with a $60\times$ oil-immersion lens ($NA = 1.4$) and excited by a collimated Ar:Kr ion laser at 488 nm . Fluorescence images were collected at 533 nm by an intensified CCD camera (Princeton Instruments) and digitally enhanced. Au electrodes appear as vertical stripes. Arrows represent the flow direction. Panel A1 and A2: Molecule A, anchored at both ends, and flexing with the flow. Panel B1 and B2: Both ends of molecule B were attached on the same electrode, with the midsegment moving freely in the buffer, showing that the attachment was specific to the thiol-modified ends. Panel C: Many HS- λ DNA molecules spanning two electrodes. The image was taken after the Mg^{2+} and NH_4Ac rinsing. Scale bar applies to all panels [24].

dye-staining step *after* the measurements to avoid inadvertent damage from dye intercalation.)

As discussed above, a crucial step in the experiment was the removal of the buffer. In preliminary experiments, we repeatedly observed a finite, semiconductor-like, history-dependent conductance after loading DNA solution and removing the buffer solution. The inset of Fig. 3 is a representative I - V curve of the buffer salt residue, $1 \times \text{TE}$. Such spurious signals are of particular concern when DNA is laid down on electrodes in a thick bundle, because the salts trapped between the DNA molecules can form conduction paths. The spurious background vanished after we adopted the following procedure. Chips containing bridging DNA molecules were carefully rinsed in 5 mM ammonium acetate (NH_4Ac , pH 6.6), a volatile buffer that can be completely removed in high vacuum. A drawback of this rinsing is that a

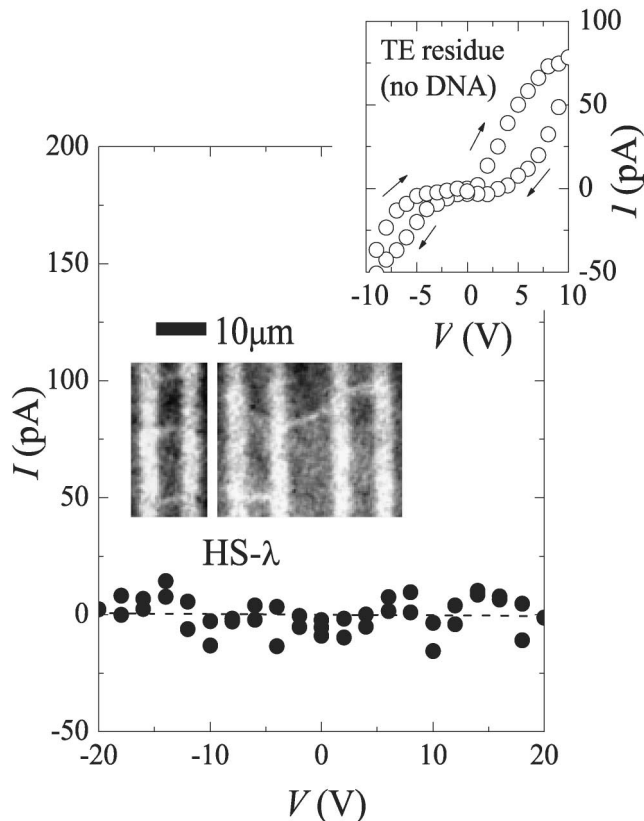


FIG. 3. The two-probe current vs voltage (I - V) curve for a sample of λ -DNA bridging two parallel Au electrodes separated by $4 \mu\text{m}$ (the sample comprises ~ 1000 molecules). The DNA was rinsed with NH_4Ac before the measurement to remove the buffer salt residue. The dashed line is a linear fit to the data. The inset shows the two-probe I - V curve for a test chip containing $1 \times \text{TE}$ buffer solution (without DNA). The chip was dried in vacuum but not subject to NH_4Ac rinsing. The observed conductance is entirely from trace TE salt residue. Both measurements were done in vacuum ($< 10^{-7}$ Torr) at 295 K. Open-circuit impedance between any two electrodes was always $\gg 10 \text{ T}\Omega$.

large fraction of the anchored DNA molecules are cut after rinsing and drying. However, rinsing in 10 mM $\text{MgSO}_4/40 \text{ mM Tris-HCl}$ (pH 8) *before* the NH_4Ac rinsing introduces Mg^{2+} ions which coat the quartz surface with weak positive charges [6]. As the negatively charged DNA molecules stick to the substrate by electrostatic interaction, damage due to NH_4Ac rinsing is minimized. Panel C of Fig. 2 shows a typical image of anchored DNA after Mg^{2+} and NH_4Ac rinsing. (Rinsing with the MgSO_4 solution also led to binding of unmodified λ -DNA. However, the yield of anchored molecules was much smaller.)

To perform the electrical measurements, unstained thiol-modified λ -DNA was attached to the Au electrodes as described above. After the final NH_4Ac rinsing, the chip was dried in the dark to avoid possible photon-induced damage. Two-probe I - V measurements were performed in moderately high vacuum ($< 10^{-7}$ Torr).

A typical room-temperature I - V curve, measured on λ -DNA spanning electrodes $4 \mu\text{m}$ apart, is shown in the main panel of Fig. 3. The voltage was swept between $\pm 20 \text{ V}$. A linear fit to the data in Fig. 3 yields $dI/dV = (-3 \pm 9) \times 10^{-14} \text{ S}$. Using a cross section of $\sim 3 \text{ nm}^2$ per molecule, and the estimated number of bridging molecules (~ 1000), we obtain the bound on the resistivity of $\rho > 10^6 \Omega \text{ cm}$ in electric fields E up to $\sim 10^4 \text{ V/cm}$. Measurements performed on several chips yielded consistent results. No current was detected within the noise level of our measurement ($\sim \pm 10 \text{ pA}$), despite sustained and deliberate efforts to improve electrical contacts between the base-pair stack of λ -DNA and Au.

Immediately after the measurements, a buffer solution with an appropriate amount of TOTO1 dye dissolved in $1 \times \text{TE}$ was loaded on the chip. By direct optical microscopy inspection of the poststained DNA, we confirmed that there were ~ 1000 DNA molecules bridging the electrodes, and thus the measurements did not destroy the DNA.

As a final check that the observed images are those of λ -DNA, we introduce DNase to digest the molecules [25]. Complete deletion of all fluorescent DNA molecules was observed. These tests leave very little room for doubt that a large number of *intact* DNA molecules were chemically bound to the electrodes during the electrical measurements.

The bound $\rho > 10^6 \Omega \text{ cm}$ in our experiment and the large bias potential applied (20 V) is at odds with many recent reports of moderately high conductivity. In some dc experiments, the DNA molecules formed bundles or networks between the microfabricated electrodes [26–28]. As noted above, high conductance may arise from residual salts trapped between the DNA strands. Contamination from other sources (C or Re) may be a problem as well in the experiment on proximity-induced superconductivity in λ -DNA [7].

Microwave absorption experiments have been used to infer that $\rho \approx 1 \Omega \text{ cm}$ at 295 K in λ -DNA [29]. The high

microwave conductivity, 10^6 times larger than our bound, is very difficult to reconcile with our data. If λ -DNA had such a high uniform conductivity, all the applied potential should fall across the contacts (~ 2 – 3 nm) to produce an E field $\sim 10^8$ V/cm, high enough to produce a large tunneling current, if not breakdown of the contact barrier altogether. This is not observed. Possibly, the microwave is detecting very short dissipative regions embedded in the insulating molecule.

Two groups recently used electrostatic force microscopy to probe the electrostatic polarization of DNA [8,9]. Our results are consistent with their conclusion that DNA is insulating. However, the electrostatic force-microscopy technique probes conductivity in the limit of weak bias potentials, so it does not rule out a transition to moderately large conductivity above a bias threshold of several volts (as reported in some experiments [4,30]). The present experiments show that insulating behavior extends to bias potentials as high as 20 V.

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- [1] C. J. Murphy *et al.*, *Science* **262**, 1025 (1993); S. O. Kelley and J. K. Barton, *Science* **283**, 375 (1999).
- [2] H.-W. Fink and C. Schonenberger, *Nature (London)* **398**, 407 (1999).
- [3] E. Braun *et al.*, *Nature (London)* **391**, 775 (1998).
- [4] D. Porath *et al.*, *Nature (London)* **403**, 635 (2000).
- [5] P. J. de Pablo *et al.*, *Phys. Rev. Lett.* **85**, 4992 (2000).
- [6] A. J. Storm *et al.*, *Appl. Phys. Lett.* **79**, 3881 (2001).
- [7] A. Yu. Kasumov *et al.*, *Science* **291**, 280 (2001).
- [8] C. Gómez-Navarro *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8484 (2002).
- [9] M. Bockrath *et al.*, *Nano Lett.* **2**, 187 (2002).
- [10] K. W. Hipps, *Science* **294**, 536 (2001).
- [11] X. D. Cui *et al.*, *Science* **294**, 571 (2001).
- [12] C. A. Mirkin *et al.*, *Nature (London)* **382**, 607 (1996).
- [13] *User Guide to DNA Modification* (Glen Research, Sterling, VA, 1999). See the references therein.
- [14] D. D. Eley and D. I. Spivey, *Trans. Faraday Soc.* **58**, 411 (1962).
- [15] In the modified nucleotide 4-thiothymidine-5'-triphosphate (S^4 -dTTP, Trilink Biotechnologies), a sulfur atom is covalently bound to the C4 site of the thymine molecule. Its close proximity to the hydrogen ion at the N3 site results in a resonant state that forms the desired thiol group.
- [16] A. G. Lezius and K. H. Scheit, *Eur. J. Biochem.* **3**, 85 (1967); T. V. S. Rao *et al.*, *Bioorg. Med. Chem. Lett.* **10**, 907 (2000).
- [17] The incorporation reaction was 100 $\mu\text{g/ml}$ λ -DNA, 0.1 mM dATP, 0.1 mM dGTP, and 0.2 mM S^4 -dTTP in $1\times$ EcoPol buffer, for a total volume of 100 μl . Five units of Klenow fragment ($3' \rightarrow 5'$ exo^-) were added. The mixture was incubated at 37 $^\circ\text{C}$ for 30 min. To stop the reaction, 1 μl of 0.5 M EDTA was added, and the mixture heated at 75 $^\circ\text{C}$ for 20 min. To exchange the buffer, the product was dialyzed against 30 ml $0.1\times$ TE at room temperature for 45 min across a 50 nm Millipore membrane.
- [18] 1% agarose gel in $0.5\times$ TBE buffer; $E = 6$ V/cm; 14 $^\circ\text{C}$; 16 h; switch time ramped from 0.1 to 40 sec.
- [19] One $5'$ overhang of the λ -DNA ends with an A, which is Watson-Crick matched to S^4 -T. The other overhang ends with G's, which are matched to C's. While sulfur-modified dCTP is available, the sulphur is remote from H atoms and the resonant thiol group cannot form. To maximize the probability of modified T's at both ends, we excluded dCTP altogether from the reaction, so that S^4 -T : G pairing could occur in place of C : G. The mutated Klenow fragment used here is incapable of detecting the mismatches.
- [20] The Klenow fragment is a large fragment of DNA polymerase I lacking the error-correcting $5' \rightarrow 3'$ exonuclease activity (this function damages the λ -DNA sticky ends). Here, we employed a mutated form of the Klenow fragment ($3' \rightarrow 5'$ exo^- , New England Biolabs) in which the proofreading $3' \rightarrow 5'$ exonuclease activity is deleted. S^4 -dTTP binds more weakly to A than unmodified dTTP [16], and hence the proofreading activity could possibly remove incorporated S^4 -dTTP.
- [21] The reaction was carried out in $1\times$ ligation buffer (New England Biolabs) at a λ -DNA concentration of 50 $\mu\text{g/ml}$. 400 units of T4 DNA ligase was added to the reaction volume of 20 μl , and incubated at room temperature for 5 h.
- [22] To clean the Au surface, freshly prepared chips were soaked in fuming HNO_3 for 30 min and further cleaned in a mixture of 18 M Ω cm deionized water, ammonium hydroxide, and hydrogen peroxide (5:1:1 by volume, 70 $^\circ\text{C}$) for 20 min. The chips were thoroughly rinsed in deionized and distilled water (DD H_2O) and kept in Ar-flushed DD H_2O for no more than 30 min before loading DNA samples. The Au surface was never allowed to dry during the process.
- [23] $1\times$ TE buffer contains 10 mM Tris [tris(hydroxymethyl)-aminomethane] and 1 mM EDTA (ethylene diamine tetra acetic acid), pH 8.0.
- [24] See videos at <http://suiling.princeton.edu/research/DNAconductance/DNAconduction.html>.
- [25] To digest the DNA, we used 10 units of DNase I (RNase-free, Roche Molecular Biochemicals) dissolved in a 10 μl reaction buffer.
- [26] A. Rakin *et al.*, *Phys. Rev. Lett.* **86**, 3670 (2001).
- [27] L. Cai, H. Tabata, and T. Kawai, *Appl. Phys. Lett.* **77**, 3105 (2000).
- [28] J. Gu *et al.*, *Appl. Phys. Lett.* **80**, 688 (2001).
- [29] P. Tran, B. Alavi, and G. Gruner, *Phys. Rev. Lett.* **85**, 1564 (2000).
- [30] H. Watanabe *et al.*, *Appl. Phys. Lett.* **79**, 2462 (2001).