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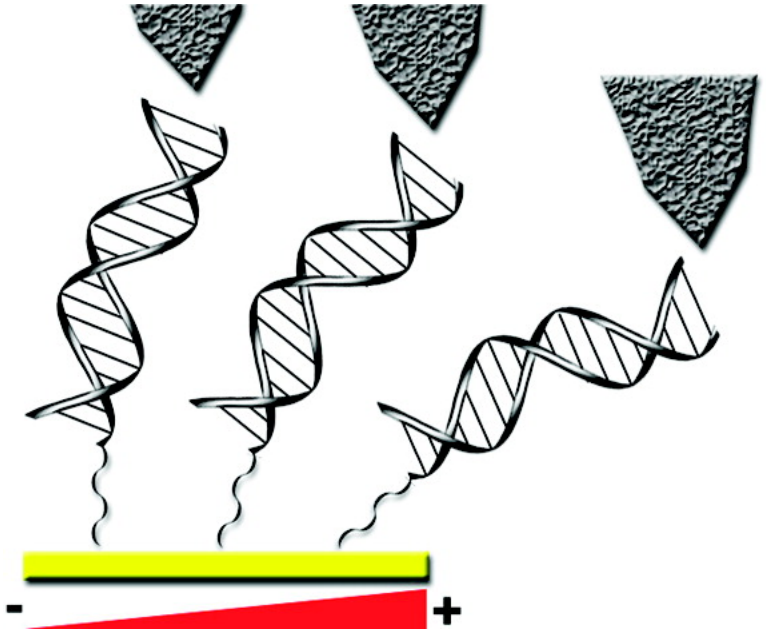

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# In Situ Scanning Tunneling Microscopy of DNA-Modified Gold Surfaces: Bias and Mismatch Dependence

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Investigations in solution have established that DNA can transport charges over significant distances.[1] The results differ depending upon the nature of the DNA probes and sequence, but all emphasize the charge transport properties of duplex DNA. On the other hand, conductivity experiments where DNA molecules are positioned between two electrodes have shown contradictory results: superconductor,[2] ohmic conductor,[3] and semiconductor.[4] Those experiments, however, lack rigorous considerations of the DNA structure and flexibility, both of which vary significantly with counterion condensation and solvent content. Disruption of the duplex structure or metal−molecule contacts may lead to loss of conductivity.[5]

To probe the electronic properties of DNA in a metal−molecule−metal assembly under physiological conditions, we have investigated thiol-modified DNA films on gold surfaces using *in situ* scanning tunneling microscopy (STM). We have shown[6] that self-assembly of duplex DNA anchored to the surface through an alkane thiol linker at the 5′ end yields well-organized films, where the DNA, at open circuit potential, forms a ∼45° angle with respect to the surface. Furthermore, these AFM studies show that an applied potential restrains the polyanionic DNA either in the upright position, for repelling negative values, or flat on the surface, for attractive positive values. By using STM at different applied potentials, then, one can directly interrogate the electronic properties of the DNA film as a function of duplex orientation. Thus, one can obtain information about the electronic states of DNA down the helical axis. Because the tunneling current is proportional to the local density of states (LDOS) of the sample,[7] images obtained at constant current do not necessarily provide the topographical morphology of the surface, but rather important insight into the LDOS of the DNA. Since these STM studies are carried out in aqueous solution (10 mM Tris-HCl, pH 7) using structurally well-characterized 15-mer oligonucleotides bound to the gold surface,[6,8] these studies provide a useful description of the electronic properties of well-defined, oriented B-form DNA in a metal−molecule−metal assembly.

DNA-modified gold surfaces were examined as a function of the applied potential and the percentage of perfectly matched (PM) duplex content in the films.[9] Figure 1 shows two DNA-modified gold surfaces (100% and 85% PM DNA) recorded at different bias potentials.[10] The features of the gold surface are clearly distinguishable when the potential applied to the surface is positive. The DNA film is not visible. Conversely, when the surface potential is negative, the gold surface is covered by features that we attribute to agglomerates of DNA. The images are stable, and the behavior is completely reversible. The measured cluster diameter varies in the range of 7−13 nm. This size range is reminiscent of hexagonal packing of DNA in crystals,[11] and indeed, $^{32}$P-labeling experiments[8] are consistent with close duplex packing on the gold surface.

The STM images shown here are consistent with AFM images at lower resolution in which films were morphologically uniform;[6] the high $Mg^{2+}$ concentrations used in fabrication neutralize phosphate backbone repulsions and permit close packing. STM images of DNA agglomerates have also been seen previously.[12]

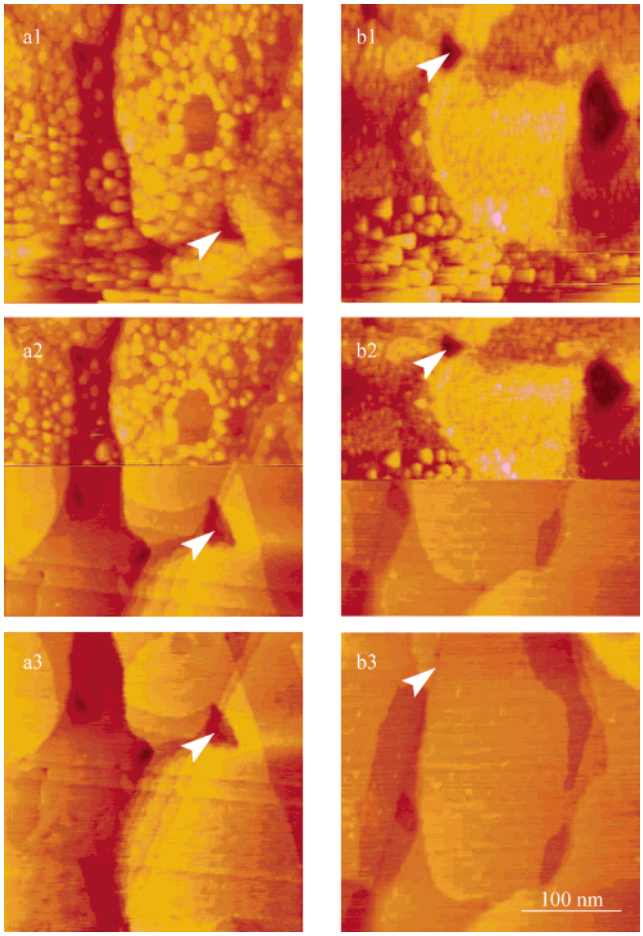


***Figure 1.*** STM images of two DNA-modified surfaces with 100% (series a) and 85% PM (series b) at different bias potentials: (1) −400 mV, (2) switching from −400 to + 400 mV during the scan, and (3) +400 mV. Arrowheads indicate fixed points on the surface. The scale bar applies to all images. Setpoint current 50 pA.

Potential-dependent behavior has been reproducibly observed for different samples with PM contents higher than 75%. As evident in Figure 1, similar potential-dependent behavior is observed for both 100% and 85% PM. For samples with lower PM content, however, the STM images do not exhibit bias potential dependence. Instead, they appear blurry and noisy (Figure 2). This blurriness suggests that the STM tip is penetrating the soft film to maintain the constant setpoint current. With high PM content, in contrast, clear images have been observed using a range of setpoint currents, 50 pA−1 nA. Thus, the presence of an internal mismatch causes attenuation of the film conductance with the DNA duplexes in the upright orientation. The mismatch dependence revealed here also



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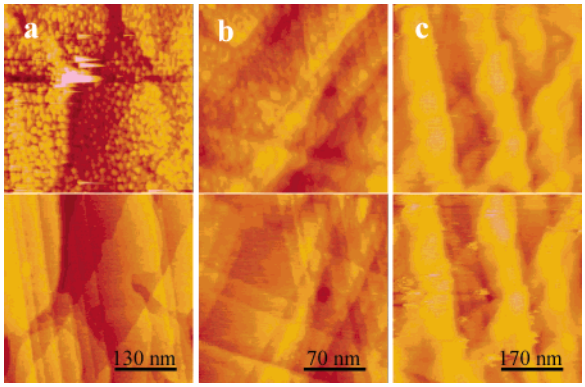


***Figure 2.*** STM images of samples containing different percentages of mismatched DNA: (a) 75% PM, (b) 70% PM, and (c) 50% PM. Top row: negative potentials; bottom row: positive potentials.

argues against ionic conductivity as a factor in the mechanism of the STM contrast.

It would be difficult to interpret these data without consideration of the contribution of the LDOS of the DNA to the electronic communication from the gold surface to the STM tip. If one considers the orientation of the DNA with respect to the STM tip, effective orbital overlap between DNA base pairs and the metal electronic states is likely when the DNA is in the upright position. In this orientation, an efficient tunneling process through the energy gap (between tip and DNA) can occur. Conversely, when the potential is more positive, the DNA lies almost parallel to the surface, and the coupling between DNA and tip decreases. In this orientation, the DNA bridge does not appear to affect tunneling between the gold and tip; in the STM images, only the features of the gold surface are visible. Interestingly, as in the AFM studies, in recording the images as a function of the applied surface potential, a hysteresis is observed, which we attribute to mechanical motion of the DNA in the film (Supporting Information). Thus, well-matched DNA films oriented in an upright position possess a non-negligible LDOS near the Fermi level of the gold surface.

Intervening mismatches in the DNA duplex, even if properly oriented, alter the STM images. Despite causing little change to the duplex DNA structure and no detectable change to the DNA film structure,[13] intervening mismatches lead to significant electronic perturbations. Theoretical calculations have shown that the states of sulfur atoms can mix with those of the alkanethiol molecules, introducing additional electronic states in the HOMO−LUMO gap,[14] yet the states of the sulfur atoms should not be affected by a mismatch inside the DNA duplex (42 Å from the sulfur atoms). In fact, for a PM content less than 70%, the LDOS of the DNA is not sufficient to keep the tip outside the film. The results with lower PM content are furthermore inconsistent with a pure tunneling mechanism over the 6.8 nm length of DNA (5.1 nm) plus linker (1.7 nm), since the intervening mismatch should not affect this long-range tunneling. Instead, results with less than 70% PM may be a consequence of the local electronic perturbation induced by the mismatch on the integrity of the $\pi$-orbital interactions that provide an electronic pathway inside the DNA. Therefore, DNA orbitals are no longer efficiently coupled, and any conductivity through DNA is turned off.

In summary, we have observed effective charge transport behavior of DNA films on gold surfaces under physiological conditions that depends sensitively upon DNA orientation, probed by varying the bias potential, and the integrity of base pair stacking, probed by varying PM content. Importantly, the mismatch acts as an electronic perturbation that exerts dramatic effects on the conductive properties of DNA, in agreement with electrochemical,[15] biochemical,[16] and photophysical[17] studies. These experiments suggest duplex DNA as a promising candidate in molecular electronics, but only in arrangements where the orbitals can efficiently overlap with the electronic states of the electrodes and the environment does not constrain the DNA in non-native, poorly stacked conformations.

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**Supporting Information Available:** Series of images at different potentials showing reversibility (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) A solution of perfectly matched (100% PM) 15-mer duplex was prepared by hybridizing with its Watson−Crick complement a 5′-derivatized[14] oligonucleotide, $SH(CH_2)_2CONH(CH_2)_6NHCOO$-5′-AGT ACT GCA GTA GCG-3′; a solution of mismatched duplexes instead contained the thiol-modified strand hybridized to a complement containing a *single* nucleotide substitution (5′-CGC TAC TG*T* AGT ACT-3′). After separate annealing, matched and mismatched duplexes were mixed to the desired % PM.
(10) A duplex DNA solution was deposited on gold−mica surfaces previously mounted on a STM fluid cell. The latter contained Ag (reference) and Pt (auxiliary) electrodes connected to a biopotentiostat that independently controlled tip and surface potentials. The tips (Pt−Ir) were insulated as described in Nagahara, L.; Thundat, T.; Lindsay, S. *Rev. Sci. Instrum.* **1989**, *60*, 3128. All images were obtained in constant current mode (50 pA setpoint), and tip potential was kept 0 V vs Ag wire using the STM controller Nanoscope III. The color code is a linear gradient from black (0 nm) to white (5 nm).
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