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A hydrogen-bonded electron-tunneling circuit reads base composition of unmodified DNA

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Abstract

Using a tunnel junction in which one electrode is guanidinium-functionalized (to trap DNA by hydrogen bonding to the backbone phosphates) and a second electrode which is functionalized with a base (to capture its complementary target on the DNA), current vs. distance curves yield an accurate measure of the base-composition of DNA oligomers. With this long tunneling path, resolution is limited to sequence blocks of about twenty bases or larger, because of the need to form a large-area tunnel junction. A shorter hydrogen-bonded path across bases will be required for DNA sequencing. Nonetheless, these measurements point the way to a new type of nanoscale sensor.

Introduction

Detection and chemical identification of one, or a few, biomolecules is currently realized by tagging targets with optical dyes¹ or redox-active labels.^{2, 3} Here, we describe an entirely new *label-free* approach to detecting molecules that display at least two distinctive target sites. The analyte molecule is trapped between two electrodes by a first reagent, attached to one electrode, that binds a first target site, and by a second reagent, attached to the second electrode, that binds a second target site. The simultaneous binding of both reagents is signaled by a distinctive tunnel-current vs. distance signature. We demonstrate this using a guanidinium-functionalized electrode (to hydrogen-bond the backbone phosphates⁴) and a second base-functionalized electrode (to hydrogen-bond the Watson-Crick complementary base.⁵)

It was previously shown that scanning tunneling microscope (STM) images of DNA bases tethered to a gold electrode are chemically-sensitive if the STM probe is functionalized with a base, giving enhanced contrast for a complementary base attached to a gold surface via a thiol linkage.⁶ We used current-distance spectroscopy of monolayers of nucleosides taken with a base-functionalized STM probe to obtain quantitative signals of base recognition.⁵ These observations raise the possibility that this phenomenon could be used to identify bases in intact DNA molecules. Ohshiro and Umezawa showed⁶ an image of a peptide-nucleic acid (PNA) molecule taken with a base-functionalized probe, but did not present data for DNA itself. STM images of PNA may be more readily obtained than STM images of DNA because the amide-gold interaction⁷ allows for electron transfer between PNA and gold. This raises the question of whether unmodified DNA could be used in tunneling measurements of this sort. We have recently shown that electrical connections can be made to unmodified DNA using a guanidinium-modified surface to hydrogen-bond to the backbone phosphates in DNA.⁴ Here,

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we assemble the complete “sandwich”: a guanidinium-functionalized electrode, trapped DNA oligomers and a base-functionalized probe (Figure 1) and show that current-distance curves are indeed remarkably sensitive to the base composition of the target oligomer.

Experimental Methods

0.1 μM solutions of oligo (dT)₄₅ or oligo (dC)₄₅ (IDT, Corvallis, OR) in 0.5 mM Tris-HCl buffer (pH 7.0) were adsorbed onto guanidinium-functionalized Au(111) electrodes to form dense, smooth monolayers as previously described.⁴ Formation of the DNA adlayer was confirmed by surface plasmon resonance and by Fourier transform infrared spectroscopy.⁴ Gold wire (0.25 mm diameter, 99.999%, Premion) was electrochemically etched using a high-frequency ac current (Appendix A) and then partially insulated using high density polyethylene⁸ in a process similar to that described for wax insulation.⁹ Probes were functionalized with 1 mM solutions of 8-mercaptoguanine (G) or 2-amino-8-mercaptadenine (2AA) in DMF for 2 to 12 h. Thiolated bases were used as received from Aldrich. Probes were rinsed with DMF and ethanol and blown dry in an N₂ stream before use. 2-aminoadenine was chosen in preference to adenine because the additional amine group forms an extra hydrogen-bond with thymine, so that the 2AA-T complex is held together with 3 hydrogen bonds, (like the G-C complex).¹⁰ Both the probe and nucleoside-functionalized substrate were submerged in buffer (0.5 mM Tris-HCl, pH 7.0) in the liquid sample cell of a scanning tunneling microscope (PicoSPM, Agilent, Chandler, AZ). The probe was advanced to a set-point tunnel current of 400 pA at a bias of 0.4 V (a conductance of 1 nS) and the decay of current recorded as the probe was withdrawn from the surface. Current on retraction was recorded using custom Labview software and a data acquisition system interfaced to the STM via a breakout box.

Results

As controls, we used bare-probes and also used functionalized probes on guanidinium-functionalized gold surfaces with no DNA adsorbed. Typical current-distance curves are shown in Figures 2a (G-probe) and 2b (2AA probe). The current decays exponentially as $i = i_0 \exp(-\beta z)$ where $\beta = 6 \text{ nm}^{-1}$ for all the control experiments. Signals in the presence of adsorbed oligomers are quite different. The three hydrogen-bonded pairs (G-C, Figure 2e and 2AA-T, figure 2d) yield signals that extend out beyond 1 nm. The 2AA-C (two h-bonds¹⁰) and G-T (two h-bonds¹⁰) signals have decayed almost completely by 1 nm. These results mirror those obtained with nucleosides as the target⁵ and are remarkably reproducible from sample to sample (Appendix B). The apparent long-range of the tunnel signals is most likely a consequence of elastic distortion of the probe,¹¹ with the hydrogen bond dependence arising from specific adhesion between the probe and target molecule.⁵

How well does an individual curve report the number of hydrogen bonds in an interaction? Curves were analyzed by integrating them, using the known withdrawal speed (133 nm/s) to obtain the total charge transferred (ΔQ) in each interaction. Histograms of the occurrence of a given ΔQ are plotted in Figure 3 for all the experimental conditions listed in Figure 2. Additional controls, using a bare probe on adsorbed DNA monolayers, are shown by the green bars in Figures 3c-f. In the absence of specific hydrogen bonding, ΔQ is almost always less than 1 pC in these conditions. Hydrogen bonded interactions give rise to much larger values of ΔQ . The spread of the signals also generates some ambiguous recordings, but, using a threshold of $\Delta Q > 2.2 \text{ pC}$ (blue shaded regions) ensures that there are no false positive reads. Thus, three-hydrogen bonded interactions may be identified with a high degree of confidence, so that a given probe can identify its complement in unmodified DNA strands.

We tested this base-recognition capability using oligomers of mixed composition (with sequences as listed on Figure 4). Histograms of ΔQ for these experiments are presented in Figure 4. The fraction of positive recognition events ($\Delta Q > 2.2$ pC) is plotted vs. the C/T ratio of the samples in Figure 5 for the two types of probe. The 2.2 pC criterion results a 50% percent success rate for G reading C and an 80% success rate for 2AA reading T (data points for the pure oligomers are on the left and right vertical axes). The recognition falls off in proportion to the reduction of target base for each of the two probes. Thus, the tunneling probe is capable of recognizing bases embedded in heteropolymers. However, a block of 20 bases was the smallest structure that gave reasonable results. Attempts to resolve smaller blocks of a given type of base did not yield reliable data and the present approach cannot resolve runs of homopolymer much less than 20 bases.

Discussion

The value of this robust electronic chemical identification is diminished by the limited resolution of the technique as implemented here. Why are single bases not resolved? It is interesting to compare the limited resolution of sequence blocks detected by current-distance spectroscopy (about 20 bases) to the resolution of STM images of DNA adsorbed onto the guanidium functionalized electrodes.⁴ The resolution in those STM images is limited to about 6nm owing to the probe pushing into the adlayer to obtain the required tunnel conductance, causing the contact area to increase in consequence.⁴ If a similar effect were at play here, then we would expect that the contact would cover 6/0.34 or roughly 18 bases. This would account for the limited resolution of our reads.

If the contact diameter (and concomitant compression of the monolayer) required to get this set-point current is really on the order of 6 nm, it would imply that the hydrogen-bonded molecular contacts make a negligible additional contribution to the tunnel current (compared to direct tunneling with an unfunctionalized probe on DNA attached to these guanidinium-functionalized surfaces⁴). We investigated this question theoretically by evaluating the conductance from a current-voltage (I-V) curve for “guanidinium-phosphate-sugar-cytosine-guanine” connected to gold contacts. The tunneling current is computed using a density functional theory (DFT) Green's function scattering method^{12, 13} based on the Landauer approach.^{14, 15} Details are given in Appendix C. The calculated conductance for “guanidinium-phosphate-sugar-cytosine-guanine” (g-p-s-c-g) is remarkably low (about a fS), smaller than the experimental set-point by several orders of magnitude. This implies that the through-bond contribution to tunneling through a single molecular chain is indeed negligible, so the probe is forced to compress against the sample in order to obtain the desired set-point tunnel current.

To understand the origin of this low conductance we separately investigated the complex bandstructure¹⁶ for the G-C base-pair and for the complete g-p-s-c-g assembly. Complex bandstructure estimates worst-case tunnel decay rates quickly, using periodic arrays of the molecular assemblies.¹⁶ Table 1 lists the structures investigated this way, together with values for β and the length L of the ‘unit cell’ of the repeated structure. An order of magnitude estimate (middle columns, Table 1) of the tunnel conductance is obtained using $G \approx G_0 e^{-\beta L}$ where G_0 is the quantum of conductance (77 μ S).¹⁶ Two striking features emerge from these calculations. First, the conductance of the G-C base pair is remarkably high, on the order of nS, a value one might expect from a sigma-bonded system.¹⁷ Second, the whole assembly shows an extremely low conductance, on the order of fS. Thus we conclude that the addition of the guanidinium-phosphate-sugar has markedly decreased the conductance of the assembly to the point where it contributes a negligible amount to the conductance of the tunnel junctions used here. To improve our estimate of the g-p-s-c-g conductance, we have computed G using β at the Fermi-level predicted by DFT calculations. At 8.32 fS (Table 1) it is still much lower

than the experimental set-point. The hydrogen-bonded molecules contribute little to the tunneling, so a compressed monolayer and a large-area tunnel junction are required to obtain the desired set-point currents.

Conclusions

In summary, we have demonstrated that tunneling with chemically functionalized contacts can read the base composition of unmodified DNA oligomers with a resolution comparable to that of ion-current readouts in nanopores.^{18, 19} It illustrates the operation of a new type of nanoscale sensor in which one electrode connects to one site on an analyte molecule, and a second electrode connects to a second site. In the present study, one of the target sites was “generic” (in the sense that every base in DNA is associated with a backbone phosphate). However, in target molecules with two distinct sites, multivalent interactions could lead to greatly enhanced specificity.²⁰ The limited resolution of the present approach makes it unsuitable for DNA sequencing applications where single-base resolution is required.²¹ It will be necessary to increase the level of through-bond tunneling substantially. Different electrode materials can be used to better align the Fermi level with molecular levels.²² Shorter tunneling paths can be formed by using recognition reagents that bond with the donors and acceptors that lie along the sides of Watson-Crick basepairs.

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Appendix A: Etching and functionalizing gold probes

Gold wire (0.25 mm diameter, 99.999%, Premion) was etched in a mixture of equal volume of concentrated HCl and ethanol (effectiveness of the mixture increases with some prior use). Etching is carried out with a 4.2 kHz square wave of 27 V amplitude with the tip immersion adjusted to achieve a current of about 0.3 mA. Probes were then partially insulated using high density polyethylene in a process similar to that described for wax insulation.⁹ Probes were functionalized with 1 mM solutions of 8-mercaptoguanine (G) or 2-amino-8-mercatpto-adenine (2AA) in DMF for 2 to 12 h. Thiolated bases were used as received from Aldrich. Probes were rinsed with DMF and ethanol and blown dry in an N₂ stream before use.

Appendix B: Reproducibility of the current-distance curves. Reproducibility of conductance vs. distance curves

A fraction of the curves taken over DNA with functionalized probes produce decay curves that resemble the controls taken on bare surfaces or with unfunctionalized probes. About half of the probes proved not to be functionalized, often because of interference from the polyethylene insulation.⁵ An additional source of variability comes from non-uniform coverage of the surface by the dense DNA adlayers. In this case, a given probe can give both positive and negative signals, as illustrated in Figure B1. The key point is that false *positive* signals are not observed.

Figure B1.

Variability in I-Z curves owing to variable DNA coverage. (A) Image of an oligomer adlayer showing an unmodified guanidinium facet on the left. (B) Sample of I-Z curves (G tip, (dC)₄₅ oligomer) taken randomly over a wide area. A fraction of the curves ("Bare") resemble the control data taken on bare guanidinium surfaces.

Appendix C Calculation of molecular conductance

I-V calculation

The molecule (guanidinium-phosphate-sugar-cytosine) was relaxed to a minimum energy configuration with a quantum chemistry code²³ using B3LYP with the 6-31+G(d,p) basis at the restricted Hartree-Fock (RHF) level. Guanine was aligned to form three hydrogen bonds with cytosine, and the hydrogen bond distance was determined by the optimization of G-C basepair using the same quantum chemistry method. The hydrogen atoms on both ends of the complete molecule are removed and sulfur is attached to form an Au-S bond with gold electrodes. The molecule is sandwiched between the pair of (111) gold slabs, and the system is periodic along the molecular axis and in perpendicular directions. The terminal sulfur is positioned above the hollow site on Au (111) surface equidistant from three Au atoms. The distance between the hollow site and sulfur was 1.95 Å. Six gold layers (4×4 in plane) were used, and the slabs are transformed into semi-infinite bulk electrode using block recursion method.^{12, 24} Similar calculations of the I-V characteristics were performed on just a G-C basepair. For this system, the S atoms are placed at ontop sites (directly above a gold atom) and the surface cell was 3×3. The self-consistent Kohn-Sham single electron states were obtained using local orbitals²⁵ with double zeta basis plus polarization orbitals (DZP) except Au (single-zeta basis plus polarization orbitals – SZP) in the local density approximation. The

current is obtained by integration over the transmission function, $I = \frac{2e}{h} \int_{\mu_L}^{\mu_R} T(E) dE$, where $T(E)$ is the transmission function and μ_L and μ_R are the Fermi energies of the left and right electrodes under the applied bias. The voltage drop is assumed to be symmetric with respect to both leads.

Cytosine-Guanine

Figure C1.

Complex bandstructure of G-C basepair. Left figure shows a conventional bandstructure with real k , and right figure shows $2\text{Im}[k]$ ($= \beta$) versus energy. The semi-elliptical curve within the bandgap region determines the tunneling decay of the most penetrating states. The quantity β_{max} is defined as the maximum decay rate in this region.

Complex band structure calculations

To understand the origin of this low conductance we separately investigated the complex bandstructure¹⁶ for the G-C base-pair and for the complete g-p-s-c-g assembly. We used the optimized structures and linked them together to form periodic structures with (-NH-) for the G-C base pair and (-CH₂-) for the g-p-s-c-g assembly. We used the plane-wave basis method²⁶ using LDA-DFT with pseudopotentials. The complex bandstructure method determines the exponential decay ($e^{-\beta L}$) tunneling parameter β as a function of energy. Figure C1 shows the β parameter in the HOMO-LUMO gap region for the G-C basepair, and Figure C2 is the complex bandstructure for the complete g-p-s-c-g molecule.

Table 1 (main text) lists the structures investigated this way, together with values for β and the length L of the 'unit cell' of the repeated structure. Two values of β are given; the first is the maximum value of β in the HOMO-LUMO gap, and the second (in parentheses) is an estimate of β at the Fermi energy corresponding to gold. The first value can be used to obtain a minimum

value of conductivity expected for any metallic contact, while the second can be used for an order of magnitude estimate specifically for gold.

An order of magnitude estimate (middle columns, Table 1) of the tunnel conductance is obtained using $G \approx G_0 e^{-\beta L}$ where G_0 is the quantum of conductance ($77 \mu\text{S}$).¹⁶ Two striking features emerge from these calculations. First, the conductance of the G-C base pair is remarkably high, on the order of 10nS even in the worst possible case using the maximum β – such a conductance is similar to a value one might expect from a sigma-bonded system.¹⁷ Second, the whole assembly shows an extremely low conductance, on the order of fS or fractions of a fS. Thus we conclude that the addition of the guanidinium-phosphate-sugar has markedly decreased the conductivity of the assembly. Complex bandstructure estimates of the g-p-s-c-g conductivity are consistent with the very low conductivity obtained from a full I-V scattering theory calculation. The I-V result of 8.62 fS (Table 1) is orders of magnitude lower than the experimental set-point. The hydrogen-bonded molecules contribute little to the tunneling, so a large-area tunnel junction is required to obtain the desired set-point currents by means of through-space tunneling, the bonded junction(s) contributing little to the overall current (but dominating the adhesion between probe and target).

Figure C2.

Complex bandstructure of “guanidinium-phosphatesugar-cytosine-guanine”. β increases significantly compared to G-C basepair.

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Figure 1.

Hydrogen-bonded tunneling pathway through DNA. An STM probe, functionalized with a base is brought into contact with a single stranded DNA oligomer adsorbed onto a guanidinium monolayer by means of specific hydrogen bonds with the backbone phosphates. The guanidinium ions are attached to a gold electrode by thiol linkages. Hydrogen bonding between the base attached to the probe and its Watson-Crick complement on the target DNA results in an extended tunnel current signal as the probe is withdrawn. All measurements were made in buffered aqueous electrolyte.

Figure 2.

I-Z curves for a guanine functionalized probe (a, c, e) and a 2-aminoadenine functionalized probe (b,d,f) over a bare guanidinium (GD) monolayer (a,b, blue curves), a 45 base oligo d(T) (c,d) and a 45 base oligo dC (e,f) adsorbed onto guanidinium-functionalized gold electrode. 3 H-bond interactions give signals that extend significantly beyond 1 nm (vertical gray lines). The results of control experiments using unfunctionalized probes are shown in green. Initial set point is 0.4 nA at 0.4V, 133 nm/s retraction speed.

Figure 3.

Histograms of charge transfer for the I-Z curves. a,c and e are for guanine functionalized probes and b,d and f are for 2-aminoadenine functionalized probes. a and b (blue bars) show data obtained on the guanidinium monolayer, c (orange bars) and d (red bars) show data obtained with oligo dT, e (brown bars) and f (red bars) show data obtained with oligo dC. Green bars show data for unfunctionalized probes. Charge transfers > 2.2 pC (blue shaded boxes) are unique to the three-hydrogen bond interactions (e and d) and so serve to identify the target base.

Figure 4. Histograms of charge transfers for the various oligomers (rows) for the two types of probe (columns). The green lines mark the 2.2 pC recognition threshold for these tunneling conditions.

Figure 5.

Plot of the fraction of tunneling curves with $\Delta Q > 2.2$ pC for a guanine functionalized probe (blue points) and a 2-aminoadenine functionalized probe (red points) as a function of the C/T ratio in target samples (sequences are listed adjacent to the points at the top of the figure). The error bars are fractions of the mean calculated from $\sqrt{N}/N$ where N is the number of positive reads. The solid lines are regressions fits.

Table 1

Estimates of electronic conductance based on tunneling theory from complex bandstructure (G-C base pair and g-p-s-c-g molecules), and from scattering theory I-V curves of the extended g-p-s-c-g and G-C molecules sandwiched between gold. The maximum inverse decay length ($\beta_{\max}$) for a unit cell length L is obtained from an infinitely repeated lattice of the structures shown here. Numbers in parenthesis are for smaller β values resulting from Fermi-level alignment with gold predicted by DFT.

Structure	Complex bandstructure estimates		Scattering theory I-V
	$\beta_{\max}(\text{\AA})$	G
<p style="text-align: center;">$L=12.8 \text{ \AA}$</p>	0.70 (0.35)	9.8 nS (870ns)	8.0
<p style="text-align: center;">$L=24.1 \text{ \AA}$</p>	1.16 (1.03)	0.056 fS (1.32 fS)	7.0
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