Electronic Supplementary Information

Direct electrical single-molecule detection of DNA through electron transfer induced by hybridization

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CONTENTS

Experimental procedure

Reagents. The reagents were of the highest grade available. De-ionized water purified with a Milli-Q water purification system (Japan Millipore, Tokyo, Japan) was used for all the experiments. All nucleotides, modified with $-(CH_2)_3SH$ linkers at their 3′ termini and purified by HPLC, were purchased from Tsukuba Oligo Service (Ibaraki, Japan). Upon receipt of the oligonucleotides, the DNAs were dissolved in a 10 mM phosphate-buffered saline (PBS) solution to prepare 100 μM DNA solutions. The solutions were divided into aliquots and stored at –20°C. Before the experiments, each aliquot was allowed to warm to room temperature and diluted to 10 μM with 10 mM PBS. For the conductance measurements of the dsDNA using unmodified Au tips, the dsDNA was prepared by combining 10 μM T_8 and S_8 ssDNA solutions. The combined solution was then heated to 70°C for 30 min and allowed to cool to room temperature for several hours.[1]

Tip Preparation. Small pieces of gold wire (0.25 mm diameter, 99.95%) were electrochemically etched in 3 M NaCl at AC 10 V. They were then washed by sonication in pure water, dipping in "piranha solution" (7:3 concentrated H_2SO_4/H_2O_2. *Caution: piranha solution reacts violently with organic compounds and should not be stored in closed containers*), and finally, thoroughly washed with pure water. The metal tips were insulated with poly(dimethylsiloxane), except for their apices, to reduce ionic and polarization currents.[2] To prepare molecular tips, the insulated metal tips were immersed overnight in the 10 μM DNA solution at room temperature. The modified tips were washed with the 10 mM PBS solution prior to use.

Sample Preparation. Ultraflat gold films epitaxially grown on mica were used as Au(111) substrates.[3] The gold substrate was immersed in the 10 μM DNA solution for 1 h. After washing with 10 mM PBS solution, the substrate was placed on an STM sample plate. The cell was filled with 0.1 M $NaClO_4$ aqueous solution for the current measurements.

Current Measurements. The tunneling current measurements were performed on an SPM 5100 with a 1 or 10 nA/V pre-amplifier (Agilent Technologies, Santa Clara, CA). Platinum wires were used as reference and counter electrodes. The DNA tip was first brought in close proximity to, but

not in contact with, the DNA-modified Au(111) surface. This procedure was achieved by applying a high set-point current (75 nA for the 8-mer DNA tip, and 7.5 nA for the 10-mer and 12-mer DNA tips) under the STM feedback control. A bias voltage of 0.2 V was employed for all the measurements. After a short delay time of 100 ms, the DNA tip was pulled up at a velocity of 20 nm/s with the feedback loop disabled, and *I*–*z* traces were recorded at a 20 kHz sampling frequency using a data acquisition unit (SL1000; Yokogawa Electric Corporation, Tokyo, Japan, or NR-500; Keyence, Osaka, Japan). This measurement was repeated approximately 3400 times for each tip. Since the direct touch of the DNA tip with the gold substrate was carefully avoided during the whole measurement procedure, we observed no phenomenon that could be associated with loss of the probe DNA from the underlying Au tips (such as disappearance of the plateaus in the *I*–*z* curves).

Data Analyses. The current histograms were constructed from the *I*–*z* traces that exhibited the plateaus, as shown in Fig. 1b. Plateaus were observed for approximately 10% of the measured *I*–*z* traces. Other traces were either simple exponential or noisier decays. The dsDNA length was estimated by assuming 3.4 Å/base-pair.[4] The length of $-(CH_2)_3SH$ was evaluated on the basis of the optimized geometry of propanethiol calculated at the DFT level with the B3LYP functional.

UV melting studies. The UV melting profile for the S_8–T_8 duplex in a 10 mM PBS solution was obtained from absorbance at 250 nm. The measurements were performed with a temperature gradient of 1.0 °C/min. The first derivative of the profile was used to estimate the melting temperature.

Table S1: Sequences of oligonucleotide used as sample and tip molecules.

Oligo name	Sequence[a]	Length (nm)[b]
T_8	5'-CAA CAA GC-3'	3.14
T_8-T	5'-CAA TAA GC-3'	3.14
T_8-A	5'-CAA AAA GC-3'	3.14
T_8-G	5'-CAA GAA GC-3'	3.14
T_8-nc1	5'-AGT TCT AT-3'	3.14
T_8-nc2	5'-TGC ACT TA-3'	3.14
S_8	5'-GCT TGT TG-3'	3.14
S_8-T	5'-GCT TTT TG-3'	3.14
T_{10}	5'-ACC AAC AAG C-3'	3.82
S_{10}	5'-GCT TGT TGG T-3'	3.82
T_{12}	5'-TGA CCA ACA AGC-3'	4.50
S_{12}	5'-GCT TGT TGG TCA-3'	4.50

[a] $-(CH_2)_3SH$ linkers were introduced at the 3' terminus. [b] The linker length was included.

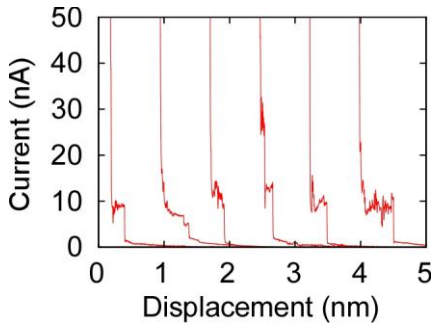

Fig. S1 *I–z* measurements of dsDNA. Measurements were performed using the unmodified Au tips and the Au substrate modified with the dsDNA composed of T_8 and complementary S_8. The plots are horizontally offset for clarity. Bias voltage: 0.2 V, initial set-point current: 75 nA.

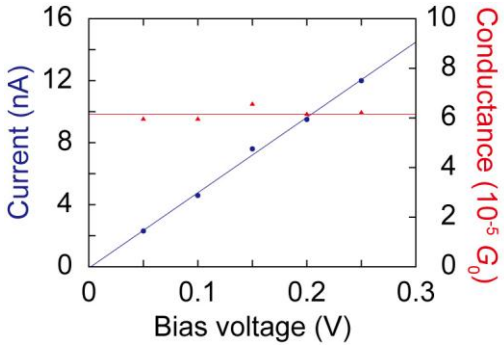

Fig. S2 Bias dependence of the peak current in the histogram (blue) and conductance (red). A T_8 DNA tip and S_8-modified sample surface were used for the measurement.

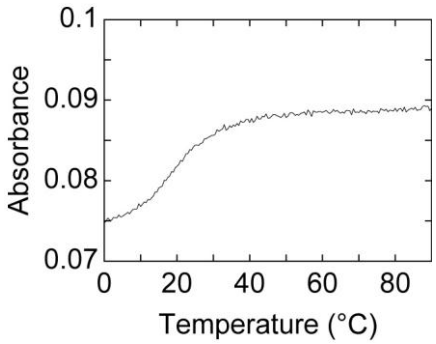

Fig. S3 UV melting profile (at 260 nm) of S_8–T_8 duplex. Concentration of the duplex: 10 μM.

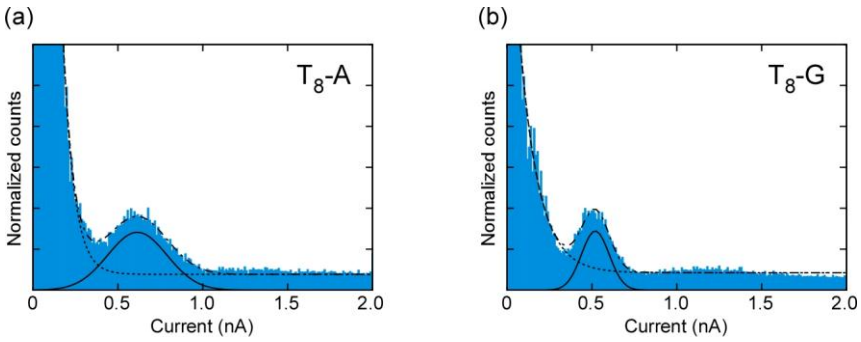

Fig. S4 Current histograms obtained using mismatch-containing DNA tips. (a) Measured using the T_8-A DNA tip and S_8 target DNA. 1693 *I*–*z* curves were used to construct the histogram. (b) Measured using the T_8-G DNA tip and S_8 target DNA. 2203 *I*–*z* curves were used to construct the histogram. The *I*–*z* measurements were performed with a bias voltage of 0.2 V and initial set-point current of 7.5 nA. Fitting components, Gaussian functions (solid line) for the current peaks, and exponential decay functions (dashed line) for the background and their sums (dashed-dotted line) are shown.

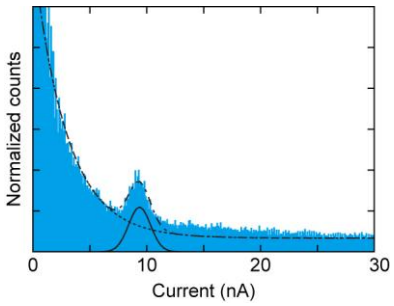

Fig. S5 Current histogram for another complementary DNA duplex. T_8-A and S_8-T were used as the tip and target DNA, respectively. 2364 *I*–*z* curves were used to construct the histogram. The *I*–*z* measurements were performed with a bias voltage of 0.2 V and initial set-point current of 75 nA. Fitting components, Gaussian functions (solid line) for the current peaks, and exponential decay functions (dashed line) for the background and their sums (dashed-dotted line) are shown.

At the position where the mismatched bases were introduced in the case of T_8-T, T_8-A, and T_8-G, the T_8–S_8 DNA duplex contains a complementary C:G base pair. *I*–*z* measurements were performed for the duplex in which the C:G pair was replaced with another complementary A:T pair in order to investigate the effect of base substitution. T_8-A and S_8-T were used as the tip and target DNA, respectively. The current histogram constructed from the *I*–*z* measurements is characterized by a prominent peak (Fig. S4). On the basis of the peak current of 0.94 nA, conductance was determined to be 4.7 nS (or 6.1×10^{-5} G_0). This value is nearly identical to conductance determined for the T_8–S_8 duplex. These results demonstrate that base substitution without mismatch causes little effect on the conductance values. It was reported that replacing C:G with a A:T base pair decreases the conductance of DNA.[1] In this study, no notable decrease by the base substitution was found. We attributed this to the shorter DNA length in the present work, which could make the effect of the A:T base less pronounced.

References

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4. W. Saenger, *Principles of Nucleic Acids Structure*, Springer-Verlag, New York, 1984.