Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information (ESI)

One-by-one single-molecule detection of mutated nucleobases by monitoring tunneling current using a DNA tip

Phuc Tan Bui,[a] Tomoaki Nishino,*[b] Hiroshi Shiigi,[b] and Tsutomu Nagaoka[b]

[a]Department of Materials Science, and [b]Department of Applied Chemistry, Osaka Prefecture University, Gakuencho 1-2, Sakai, Osaka 599-8570, Japan.

*Corresponding author. E-mail: nishino@chem.osakafu-u.ac.jp

Contents

Table S1 Sequences of oligonucleotides used as sample and tip molecules

Oligo name	Role	Sequence[a]
T_8	tip	5′-GCT TGT TG-3′
S_8	sample	5′-CAA CAA GC-3′
S_8-T	sample	5′-CAA TAA GC-3′
S_8-m^5C	sample	5′-CAA m^5CAA GC-3′
S_8-$m^{N6}A$	sample	5′-CAA Cm^{N6}AA GC-3′
S_8-nc	sample	5′-AGT TCT AT-3′

[a]For each oligonucleotide, a q–$(CH_2)_3$SH linker was introduced at its 3′ terminus.

Experimental procedures

Reagents. The reagents were of the highest grade available. Deionized 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.

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.

Tip Insulation. The etched metal tips were insulated with poly(dimethylsiloxane) (PDMS) according to the reported procedure.[1] Briefly, the tip terminal was immersed into the PDMS solution and pulled back slowly at the rate of 0.05 mm/s. The PDMS layer was cured at 150°C for 15 min (see Fig. S1 for the scanning electron micrograph of the insulated tip). The apparent radii of the uninsulated parts of the tip apices were estimated to be approximately 20 μm based on

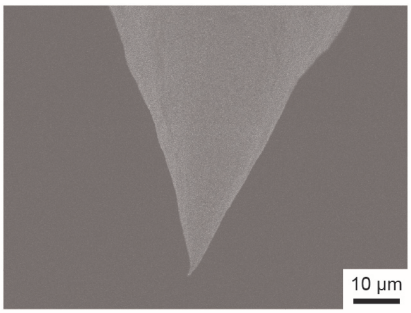

Fig. S1 Scanning electron micrograph of the unmodified Au STM tip insulated with PDMS.

electrochemical measurements.[1] The background currents, which could be caused by ionic and polarized currents, measured with these insulated tips in a 0.1 M $NaClO_4$ aqueous solution were almost the same as those detected in air.

Tip Modification. The insulated metal tips were immersed overnight in the 10 μM DNA solution at room temperature. Full, or at least highly dense, monolayer formation on the tip is expected under this condition. The sample surface was, on the other hand, modified with the target DNA at a low coverage, which is important to create a single-molecule junction. 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.[2] 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 (Agilent Technologies, Santa Clara, CA). Platinum wires were used as reference and counter electrodes, and the surface potential of the Au(111) substrate was held at its rest potential during the measurement under potential control. Before current measurements, the STM instrument was stabilized according to the reported procedure[3] to suppress unwanted thermal drift of the STM scanner. First, the DNA tip was brought in close proximity to, but not in contact with, the DNA-modified Au(111) surface by applying a high set-point current with a bias voltage of 0.2 V under the STM feedback control. In current–distance (*I*–*z*) measurement, a set-point current of 75 nA was employed. 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 of STM disabled, and *I*–*z* traces were recorded. In current–time (*I*–*t*) measurements, a set-point current of 20 nA was used, and *I*–*t* traces were recorded with the DNA tip held stationary by disabling the feedback loop for 1 s. Both the *I*–*z* and *I*–*t* traces were recorded at a 20 kHz sampling frequency using a data acquisition unit (SL1000, Yokogawa Electric Corporation). The current measurements were repeated using independently prepared tips and sample surfaces to

ensure the reproducibility. For each target DNA, at least three sample surfaces were used. The measurements were performed with six or seven DNA tips for each sample surface. In total, at least 20 tips were used for a given target DNA. Multiple current histograms (at least three) were constructed from the data sets obtained with every sample surface. The standard deviations depicted as error bars in Fig. 4a were determined by the peak values of these histograms.

References

1. M. Kuroda and T. Nishino, *Rev. Sci. Instrum.*, 2011, **82**, 063707.

2. P. Wagner, M. Hegner, H. J. Guntherodt and G. Semenza, *Langmuir*, 1995, **11**, 3867.

3. T. Nishino, *ChemPhysChem*, 2010, **11**, 3405.