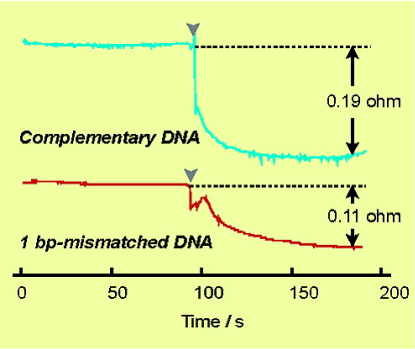
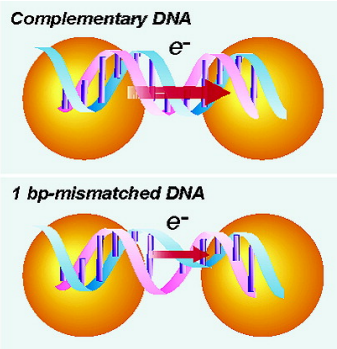
Communication

Label-Free Electronic Detection of DNA-Hybridization on Nanogapped Gold Particle Film

Hiroshi Shiigi, Shiho Tokonami, Hidetaka Yakabe, and Tsutomu Nagaoka

J. Am. Chem. Soc., **2005**, 127 (10), 3280-3281• DOI: 10.1021/ja0445793 • Publication Date (Web): 17 February 2005





More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 02/17/2005

Label-Free Electronic Detection of DNA-Hybridization on Nanogapped Gold Particle Film

Hiroshi Shiigi,† Shiho Tokonami,† Hidetaka Yakabe,‡ and Tsutomu Nagaoka*,†

Research Institute of Advanced Science & Technology, Osaka Prefecture University, 1-2 Gakuen, Sakai 599-8570, Japan, and Faculty of Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube 755-8611, Japan

Received September 8, 2004; E-mail: nagaoka@riast.osakafu-u.ac.jp

DNA microarray chips are designed to facilitate large-scale nucleic acid analysis enabling the simultaneous analyses of a number of DNA sequences.[1,2] However, the instrumentation of these chips usually requires the use of sophisticated optical devices and elaborately designed fluorescence-labeling molecules, which often make these techniques unstable and costly in practical applications. In comparison to these techniques, molecular electronic devices promise a straightforwardness in signal interfacing and in the fabrication of nanosized integrated systems. We have assembled a nanogapped electrode system using a gold particle and successfully applied this system to a label-free electronic DNA sensing device.

To construct an electronic sensing device, the conductance of a receptor-target molecule complex has a crucial importance. Although native double-strand (*ds*) DNA had been regarded as an insulator, some recent studies suggested that π overlapping between adjacent base pairs makes *ds*DNA superconductive,[3] conductive,[4] or semiconductive.[5,6] On the basis of the conductivity mechanism, some attempts of detecting DNA hybridization have recently been made using a multistep process.[7,8] However, no reports about the on-chip electronic analysis of DNA hybridization have appeared in the literature. In this communication, we discuss the fabrication of such a system.

Model illustrations of a nanogapped electrode are given in Figure 1; a Au particle is adsorbed on both the glass and Pt electrode, and a bridge molecule (decanedithiol) was used to make a gap between each particle. In the present study, a gold nanoparticle (ca. 80 nm in diameter) was prepared by the literature procedure:[9] A quantity of 10 mL of 3.0% citric acid as a reducer was added to 200 mL of 0.028% aqueous chloroauric acid, and the mixture was stirred at 80 °C for 20 min to produce a dispersion of 0.14 g L^{-1}. The Au nanoparticle film was prepared as follows: An interdigital Pt microelectrode with a 5-μm gap (NTT-AT, Japan; Figure 1Aa) was electrochemically cleaned by repeated potential sweeps (~−0.25 to +1.3 V versus Ag|AgCl) in 0.1 M H_2SO_4.[6] The electrode was immersed in 5 mM ethanolic decanedithiol for 30 min (Figure 1Ab), then immersed in the Au dispersion at 22 ± 1 °C for 10 min to anchor the Au particle on the electrode (c). The modified electrode was immersed again in the dithiol solution (d) and then in the dispersion (e) to obtain a film on the glass. Then, the electrode was immersed in the dithiol (f). A thorough rinsing with ethanol followed each thiol-dipping procedure. The procedures (d → e and e → f) were repeated until a desired resistance (~200 Ω) was obtained (g). Although the algebraic estimation suggests that the complete coverage of the glass surface with the Au particle in this fashion requires ~30 dipping sequences, five times were enough to obtain a film ready for use. Adsorption of particles on the glass, found at an early stage of the dipping by SEM, would have accelerated the film formation. AFM and SEM observations showed

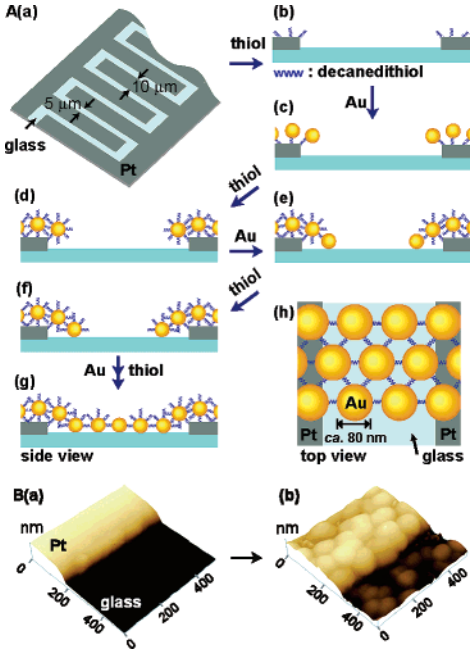


Figure 1. (A) Sensor preparation procedures (a−g); (a) interdigital electrode (one pair on the chip; each row consists of a 65-tooth comb), and (g) side view and (h) top view of Au nanoparticle film. (B) AFM images of the electrode (a) before and (b) after the modification with Au particles.

that the film was composed of evenly spaced monolayered particles (Figure 1B).[10−13] The resistivity of the film (45 Ω cm) under a N_2 atmosphere was comparable to the value (20 Ω cm) reported for nonanedithiol.[12]

The particle was modified with thiolated probe DNA by applying 5 μL of a TE buffer (pH 7.4; ionic strength, 1.0) containing 500 pmol of a 12-base single-strand (*ss*) DNA (5′-HS-TCT CAA CTC GTA-3′; Nisshinbo, Japan) on the Au film, and then the electrode was allowed to stand for 30 min. The electrode was thoroughly rinsed with a TE buffer to remove the excess probe and dried under a N_2 stream. To measure the base resistance, 1 μL of a TE buffer was applied on the film. After the resistance became steady, a 5 μL TE buffer involving sample *ss*DNA (500 pmol) was added over the film for hybridization. The resistance was monitored at 25 °C with a digital multimeter (Hewlett-Packard model 34401A, applied current: 1 mA) using a standard two-probe configuration. The sensor was immersed in water at 80 °C for 3 min to denature the hybridized *ss*DNA for the next measurement. All the experiments were repeated at least 3 times, and the resistance values reported for the DNA sensing had an accuracy of ±0.01 Ω.

The film resistance depended on the dithiol concentration and the length of its alkyl chain as well as the number of immersion sequences.[10−13] An increase in the decanedithiol concentration led

† Osaka Prefecture University.
‡ Yamaguchi University.

10.1021/ja0445793 CCC: $30.25

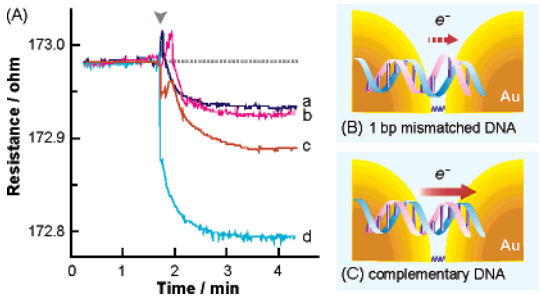


Figure 2. (A) Resistance changes by hybridization of the probe with (a) 11-base (3′-CCC CCC CCC CCC-5′), (b) 4-base (3′-AGA GTT AAC TCT-5′), (c) 1-base (3′-AGA GTT GAG CCT-5′) mismatched sample strands, and (d) complementary oligonucleotide (3′-AGA GTT GAG CAT-5′); sample addition is indicated by the arrow mark. Model illustrations for electron transfer with (B) 1-bp mismatched and (C) complementary strands.

to a decrease in the film resistance, which leveled out at ~173 Ω at 5 mM. The decrease would be attributed to the formation of a totally conducting network and therefore led to less Au-thiol immersion cycles to obtain a sensor film.[13] The presence of a N_{1s} (399.0 eV) peak in X-ray photoelectron spectroscopy confirmed the immobilization of the DNA probe on the Au surface.[14] The result also suggests that the probe (ca. 4 nm in length) would be overlaid between two Au particles, which are separated ca. 1.3 nm with dithiol (see Figure 2B,C).

Upon sample addition, a resistance decrease was immediately observed with *S*/*N* ratios above 40, followed by a steady state within 2 min (Figure 2A). The magnitude of the response depended on the number of the mismatched base pair (bp) in DNA, and the largest among the samples was the complementary strand (0.19 Ω). An increasing number of mismatches led to a decrease in the magnitude, and the 11-bp mismatched DNA showed the smallest response (0.05 Ω). It should be noted here that the resistance change behaved in a nonlinear fashion with respect to the number of mismatches. The behavior can amplify the presence of a 1-bp mismatch and be characterized as an important diagnostic advantage on detecting single nucleotide polymorphism (SNP). A linear response was found in a DNA concentration range of 5−100 μM (25−500 pmol). We also carried out experiments using probes with five different sequences, and the obtained results were similar to that shown in Figure 2.

The bare electrode had resistances of 120 MΩ and 390 kΩ in dry N_2 and a TE buffer (pH 7.4, ionic strength, 1.0). Modification with a Au film significantly decreased the resistance to 173.18 and 172.88 Ω in N_2 and the buffer, respectively. Therefore, the large decrease (120 MΩ to 173.18 Ω) can be attributed to electron transfer between the Au particles. By assuming that as an equivalent circuit, three resistors responsible for the leak (120 MΩ), electron transfer, and buffer ion migration are connected in parallel and that the total migration current does not change with the film formation, we evaluated the film and migration resistances to be 172.96 Ω and 391 kΩ, respectively, in the buffer. Accordingly, ionic migration has only minor effects on DNA sensing.

Therefore, the resistance changes in Figure 2 can be due to the conductivity of the *ds*DNA wire, which can be explained in terms of π overlapping between adjacent base pairs.[4−6,15,16] The presence of a mismatch would produce a defect for electron transfer, which arises in the localization of electrons to reduce the electron transfer rate. While the resistance change significantly depended on the number of mismatches, it was virtually independent of the location where such a 1-bp defect was along the strands (<0.01 Ω). Furthermore, Figure 2A shows that the resistance change becomes constant when the strands carry >4 mismatches.

Dekker et al. reported that the conductivity of *ds*DNA when measured on an 8-nm-gap electrode fell into a semiconductor region, while the use of a 40-nm-gap led to an extremely high resistance (more than 10 TΩ).[5,17] Their results suggest that a much narrower gap (≪8 nm) would be required to detect a small resistance change caused by a mismatch. Consequently, it can be emphasized here that our straightforward technique adopted for preparing a smaller gap (1.3 nm) can successfully be applied to detect such a small change. However, the use of a bridge molecule shorter than decanedithiol led to a smaller sensitivity because of a substantial decrease in the base resistance. To obtain the highest sensitivity and reproducibility for a particular length of an oligonucleotide sample, some parameters, such as the length of the thiol molecule and size of the Au particle, can be optimized.

To make the conduction mechanism clearer, we have studied a dithiolated 12-bp probe, both ends of which were modified with thiol. In this case, the dithiolated *ss*DNA functions as both the bridge and probe molecules. With this film, the complementary strand gave a resistance change of 0.58 Ω, which was much greater than that for the monothiolated probe (0.19 Ω). The greater response suggests that the dithiolated probe is located more efficiently across Au particles, while some of the singly thiolated probes could not efficiently participate in electron transfer due to the lack of a covalent affinity to a Au particle at the unmodified end.

To verify that the hybridization causes the resistance changes, we studied the effect of DNase I (from *Bovine pancreas*), an enzyme that randomly breaks down DNA strands at their phosphodiester bonds.[5] After hybridization with the complementary strand in the same manner as before, 10 μL of 10 mg/mL DNase I with 5 mM $MgCl_2$ was added on the film. As decomposition by the enzyme occurred, the resistance recovered in 40 min to the value before hybridization. Little resistance change observed in the absence of Mg^{2+} indicated that the recovery was due to the enzymatic decomposition of DNA. Although the ionic strength of the solution decreased ca. 7 times due to the dilution caused by the addition of the enzyme solution, the resistance does not change appreciably in this range of the ionic strength (<0.05 Ω).

In summary, a nanogapped particle film has been successfully applied for fabricating a label-free DNA sensing device, which can amplify a single base pair mismatch required in an SNP diagnosis.

References

(1) Storhoff, J. J.; Lazaorides, A. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L.; Schatz, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4640.
(2) Shalon, D.; Smith, S. J.; Brown, P. O. *Genome Res.* **1996**, *6*, 639.
(3) Kasumov, A. Yu.; Kociak, M.; Guéron, S.; Reulet, B.; Volkov, V. T.; Klinov, D. V.; Bouchiat, H. *Science* **2001**, *291*, 280.
(4) Fink, H.-W.; Schönenberger, C. *Nature* **1999**, *398*, 407.
(5) Porath, D.; Bezyadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635.
(6) Okahata, Y.; Kobayashi, T.; Tanaka, K.; Shimomura, M. *J. Am. Chem. Soc.* **1998**, *120*, 6165.
(7) Takenaka, S.; Yamashita, K.; Takagi, M.; Uto, Y.; Kondo, H. *Anal. Chem.* **2000**, *72*, 1334.
(8) Park, S.-J.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1503.
(9) Turkevich, J.; Garton, G.; Sevenson, P. C. *J. Colloid Sci.* **1954**, *9*, 26.
(10) Sato, T.; Ahmed, H.; Brown, D.; Johnson, B. F. G. *J. Appl. Phys.* **1997**, *82*, 696.
(11) Zamborini, F. P.; Leopold, M. C.; Hicks, J. F.; Kulesza, P. J.; Malik, M. A.; Murray, R. W. *J. Am. Chem. Soc.* **2002**, *124*, 8958.
(12) Han, L.; Daniel, D. R.; Maye, M. M.; Zhong, C. J. *Anal. Chem.* **2001**, *73*, 4441.
(13) Shiigi, H.; Yamamoto, Y.; Yakabe, H.; Tokonami, S.; Nagaoka, T. *Chem. Commun.* **2003**, 1038.
(14) Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 8916.
(15) Richter, J. *Physica E* **2003**, *16*, 157.
(16) Arkin, M. R.; Stemp, E. D. A.; Holmin, R. E.; Barton, J. K.; Hörmann, A.; Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475.
(17) Storm, A. J.; van Noort, J.; de Vries, S.; Dekker, C. *Appl. Phys. Lett.* **2001**, *79*, 3881.

JA0445793