Direct Attachment of Double-stranded DNA to Gold Surface for Preparation of Nano-structured Devices

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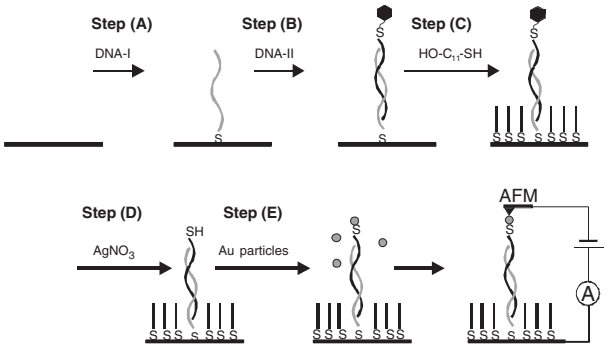
(Received April 9, 2004; CL-040389)

To the 5′-ends of two complementary oligonucleotides, 5′-mercapto-5′-deoxythymidine was introduced and reacted with the surface of either gold plate or colloidal gold particle. Well-defined $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composite was fabricated in self-assembled monolayer (SAM) of 11-hydroxy-1-undecanethiol. The current through this composite was measured by conductive probe atomic force microscopy (CP-AFM).

DNA is one of the most promising candidates for building blocks in bottom-up nanotechnology. Its molecular size, topological specificities, and self-assembling properties satisfactorily fulfill the requirements for fabrication of various molecular devices. For the communication with external systems, DNA is usually attached to electrodes or nanoparticles as interfaces.[1,2] In most cases, a thiol group was bound to DNA via alkyl chain (e.g., hexamethylene), and reacted with gold surface to form Au–S linkage (see Figure 1B). A number of elegant nano-devices have been obtained by this strategy. However, the alkyl chains used for this indirect attachment often diminish the transfer of signals from DNA to gold interface (and vice versa),[3] and thus their removal is desirable. In this paper, DNA is directly attached to gold plates and gold nanoparticles by using 5′-mercapto-5′-deoxythymidine (*T), as depicted in Figure 1A. Formation of well-defined $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composites is clearly evidenced by quartz-crystalline microbalance (QCM), scanning tunneling microscopy (STM), and conductive probe atomic force microscopy (CP-AFM).[4] These composites are considerably more conductive than the corresponding composites involving hexamethylene linker.

The phosphoramidite monomer of thymidine analog *T having 5′-SH was synthesized according to the literature,[5] and incorporated to the 5′-end of DNA on an automated synthesizer. The two 20-mer DNAs used in the present study are complementary each other: DNA-I, 5′-*TCG CGC TGG CGC GAG CCC CA; DNA-II, 5′-*TGG GGC TCG CGC CAG CGC GA. The melting temperature of the duplex between them in PBS(-) buffer is about 80 °C, as determined from the absorbance change at 260 nm. The colloidal solutions of gold particles were purchased from Sigma, and the averaged particle size is 5 nm unless noted otherwise. Water was purified by a Millipore purification system Milli-Q.



Scheme 1. Procedure for the preparation of $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composites. The closed hexagonals are the trityl groups protecting the 5′-end of DNA-II.

The nano-structured composites were fabricated as shown in Scheme 1.[6] First, a gold plate was cleaned with ethanol and thoroughly rinsed with water. This plate was immediately immersed in 1.0 μM (M = mol dm^{-3}) solution of DNA-I in PBS(-) buffer (step (A)). After 2 h, the plate was rinsed with water and subsequently immersed for 1 h in 1.0 μM solution of DNA-II which is

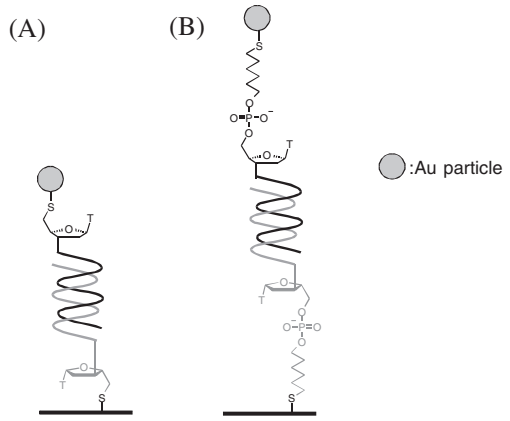


Figure 1. Direct attachment (A) and indirect attachment (B) of DNA to gold surface.

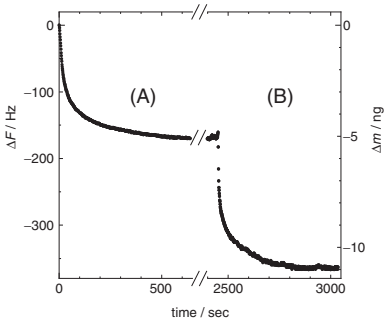


Figure 2. Change of QCM frequency responding to (A) the binding of DNA-I to the gold electrode and (B) the hybridization of 5′-protected DNA-II with the DNA-I bound to the electrode. Conditions: $[DNA\text{-}I]_0 = [DNA\text{-}II]_0 = 125$ nM in pH 7.4 PBS(-) buffer at 20 °C.

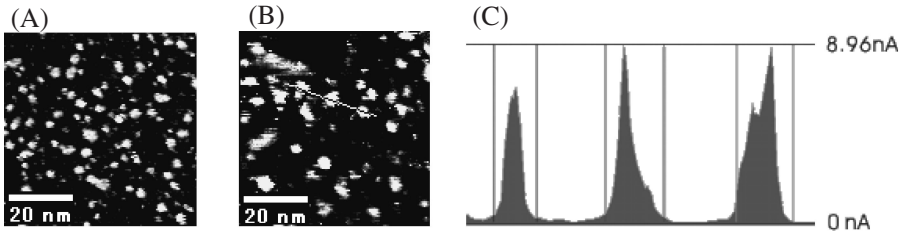


Figure 3. STM image (A) and CP-AFM image (B) of the $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composite. In (C), the current profile along the line in (B) is shown.

protected at the 5′-end with a trityl group (step (B)). Then, the gold plate was rinsed and kept in 1 mM solution of 11-hydroxy-1-undecanethiol for 2 days to cover the surface with its SAM (step (C)). The trityl group was removed from DNA-II by keeping the plate in 1 M $AgNO_3$ solution for 0.5 h (step (D)). The sample was rinsed with water and incubated for 2 days in a colloidal solution of gold particles (step (E)). Finally, the sample was rinsed again and a CP-AFM measurement was carried out on an SPA300HV unit using Au-coated AFM Probe SI-AF01A (from Seiko Instruments Co.).

Completion of the step (A) and the step (B) in Scheme 1 was confirmed by QCM (Affinix Q system, INITIUM INC). When a gold electrode for QCM was soaked in PBS(-) solution of DNA-I (125 nM), the frequency decrease (ΔF_A) was about 175 Hz (the part A in Figure 2). This change corresponds to the mass increase (Δm) of 5.3 ng (0.86 pmol of DNA-I). The elecrode was then rinsed and immersed in a PBS(-) solution of 5′-end protected DNA-II. As shown in the part B of Figure 2, the frequency further decreased and saturated at about $\Delta F_B = 195$ Hz ($\Delta m =$ 5.9 ng). The amount of 5′-protected DNA-II bound to the plate is 0.91 pmol, which is identical with the amount of DNA-I bound in the step (A) within experimental error. Thus, these complementary DNAs almost completely hybridize each other even on the gold surface. From the values of ΔF_A and ΔF_B, each of the double-stranded DNA molecules occupies around 10 nm^2 on the surface of the gold electrode.

Figure 3A shows the STM image of $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composites thus obtained. The size of bright spots is in fair agreement with the size of gold particles used (5 nm). When another colloidal gold solution (the averaged particle size 10 nm) was used, bright spots of the corresponding size were observed as expected. The possibility that these particles are simply adsorbed on the surface of gold plate (covered with the SAM) is ruled out by the following two control experiments. First, no bright spots were observed by STM, when the gold plate was directly (without steps (A) and (B)) immersed in 1 mM solution of 11-hydroxy-1-undecanethiol for 2 days and then was incubated for 2 days in a colloidal solution of gold particles. Second, the DNA-I/$Au^{(plate)}$ system, obtained by step (A), never bound the gold particles, when it was directly (without step (B)) incubated in the colloidal gold solutions. In order to form the present nanostructured composite, (i) covalent binding of DNA-I to the gold plate, (ii) hybridization of DNA-II to the DNA-I bound to the gold plate, and (iii) covalent binding of DNA-II to the gold particle are inevitable. CP-AFM also provided clear images of the gold particles of diameter 5 nm (see Figures 3B and 3C). It is noteworthy that this $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composite shows considerable conductivity. When the bias voltage was 0.05 V, the current through the composite was about 7 nA (±20%).[7] The current was hardly altered even when the composite was stored under P_2O_5 for 2 days.

For the purpose of comparison, similar composite was prepared by indirect attachment of DNA to the surface of gold plate and gold nanoparticle. Commercially available hexamethylene linker was used for the fabrication (see Figure 1B). However, the current through this composite was around 10-fold smaller than that observed on the direct attachment. Apparently, the alkyl linker suppresses the conduction at both the DNA/$Au^{(plate)}$ and the $Au^{(nanoparticle)}$/DNA junctions.

In conclusion, DNA molecules are directly attached to gold plates and gold nanoparticles by using 5′-mercapto-5′-deoxythymidine as the terminal nucleoside, and well-defined $Au^{(nanoparticle)}$/DNA-duplex/$Au^{(plate)}$ composites are fabricated. These composites are more conductive than the composites involving hexamethylene linker, and promising for further applications.

The authors thank Dr. Takao Ishida of National Institute of Advanced Industrial Science and Technology for valuable comments on CP-AFM measurement. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Published on the web (Advance View) May 17, 2004; DOI 10.1246/cl.2004.700