doi:10.1246/cl.2010.1084

Published on the web September 4, 2010

Isolation of Gold Nanoparticle/Oligo-DNA Conjugates by the Number of Oligo-DNAs Attached and Their Formation of Self-assembly

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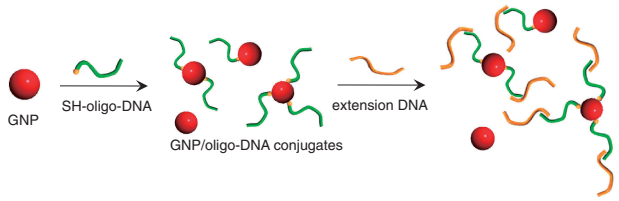
A simple and conventional method to isolate gold nanoparticle (GNP)/oligo-DNA conjugates having defined number of oligo-DNAs by gel electrophoresis was developed using extension oligo-DNAs. Formation of self-organized assemblies of GNPs in two different sizes using the conjugates having one or two complementary oligo-DNAs was demonstrated. This method to prepare GNP/oligo-DNA conjugates with controlled number of DNA strands should be useful to build up functional nanoarrays of GNPs on DNA scaffolds.

Colloidal nanocrystals such as metal and semiconductor have unique properties and attract wide interest especially in nanoscience and nanotechnology. For example, gold nanoparticles (GNPs) have specific optical and electrical properties, such as surface plasmon resonance, due to their large surface areas, which are very different from the properties of bulk state.[1] The spatial arrangement of GNPs is expected to offer emerging new properties which are not observed in distributed state. Therefore, the organization of GNPs in higher-order, 1 to 3 dimensions, is an objective of bottom-up nanotechnology.[2]

On the other hand, DNA is a useful and versatile molecule to construct highly ordered self-organized structures in nanoscale. Many approaches to construct ordered or well-designed nanostructures using DNAs have been performed.[3] Recently, some groups succeeded to construct GNP arrays using DNA scaffolds.[2a,4–7] In these approaches, however, spatial fitting of GNP on the DNA scaffolds or post-spotting of GNPs onto already-prepared DNA nanostructure by coordination bonds of thiol groups was used for addressing of the GNPs. To construct more complicated arrangements of GNPs on well-designed DNA nanostructures, control of number of DNAs introduced onto a GNP is desired. However, the control of number of oligo-DNAs introduced onto a GNP has not been very successful, except for a few exceptions.[5–7] Although GNP bearing a few relatively long DNA strands could be isolated by electrophoresis,[5] the long DNA is sometimes an obstacle for free design of ordered nanostructure. GNP/oligo-DNA conjugate having defined numbers of short oligo-DNAs attached should be useful for construction of GNP nanoarrays but is still not easy to be prepared.

Here we report a simple and conventional method to isolate GNP/oligo-DNA conjugates having defined number of relatively short oligo-DNAs attached. Moreover, we demonstrated the formation of specific aggregations of two different sizes of GNP by the complementary hydrogen bonding of oligo-DNAs introduced.

GNPs with 5 and 10 nm diameters (GNP5 and GNP10) were reacted with bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium (BSPP) to modify the surface of GNP with phosphine. After purification by centrifugal filtration, the concentration of the GNP was determined by UV–visible spectrum using an absorbance coefficient value reported previously.[8] Aqueous solutions of two different 5′-thiol-modified 40mer single-strand oligo-DNAs, SH40a and SH40b, having complementary sequence were reacted with phosphined GNP5 and GNP10, respectively, in equivalent amount (Scheme 1) to give GNP/oligo-DNA (GNP5/SH40a and GNP10/SH40b) conjugate solution (for reaction details and the sequences of oligo-DNAs, see Supporting Information).[9]



Scheme 1. Schematic illustration for preparation of GNP/oligo-DNA conjugates and the chain extension.

To facilitate isolation of the GNP/oligo-DNA conjugates by electrophoresis, chain extensions by hybridization were carried out using two longer (85mer) single-strand DNAs (extension DNAs), E85a and E85b, whose 3′ termini 15 residues are complementary to the 3′ termini 15 residues of SH40a and SH40b, respectively (Scheme 1).

The results of agarose gel (3%) electrophoresis (100 V, 90 min, 10 °C) for isolation of the conjugates are shown in Figure 1. The colloidal GNP solution had red color and could be visualized without staining. The GNP5 and GNP10 showed almost clear single bands (Lane 1 and Lane 3). On the other hand the GNP5/SH40a+E85a (Lane 2) and GNP10/SH40b+E85b (Lane 4) were separated into a band GNP itself and several clear bands of lower mobility than GNPs used for preparation of each conjugate. This suggested the obtained GNP/oligo-DNA conjugates could be separated by the difference of number of oligo-DNAs introduced per GNP. When extension oligo-DNAs (E85a and E85b) were not used, GNP5/SH40a and GNP10/SH40b were not separated clearly.[9] Three bands observed for Lane 2 can be regarded as GNP5 itself and GNP attaching one and two oligo-DNA strands (SH40a + E85a), from bottom to top, respectively. Similarly, four bands observed for Lane 4 can be regarded as GNP10 itself and GNP attaching one to three oligo-DNA strands (SH40b + E85b), from bottom to top, respectively. The reason why fewer bands observed for GNP5/SH40a+E85a than GNP10/SH40b+E85b is probably that the absorption coefficient estimation was not accurate and that the amount of SH40a added to GNP5 was smaller than intended.

After electrophoresis, each band containing GNP5/SH40a+E85a and GNP10/SH40b+E85b was extracted from

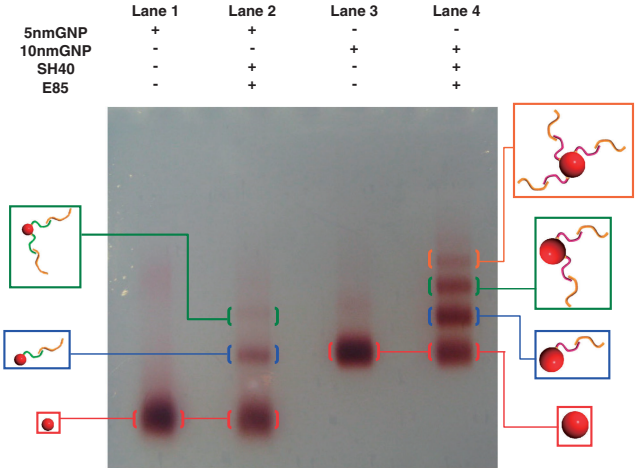


Figure 1. The results of gel electrophoresis for isolation GNP/oligo-DNA conjugates: Lane 1, GNP(5 nm); lane 2, GNP5/SH40a+E85a; lane 3, GNP(10 nm); lane 4, GNP10/SH40b+E85b.

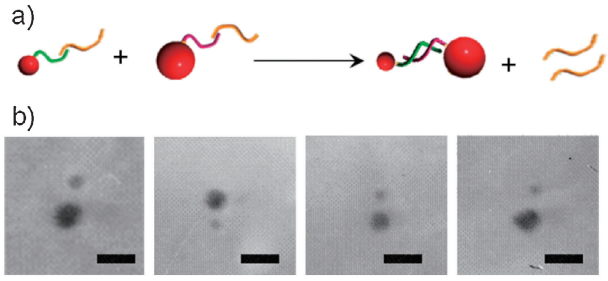


Figure 2. a) Schematic illustration for the formation of GNP aggregate by hybridization of **1a** and **1b**. b) TEM images of the aggregation of **1a** and **1b**. The scale bars are 20 nm.

the gel using a glass fiber filter (GF/C, Whatman) by method reported previously.[7] The obtained solutions containing GNP5 attaching 1 or 2 oligo-DNA strands, GNP5/$(SH40a)_1$ (**1a**) or GNP5/$(SH40a)_2$ (**2a**), and GNP10 attaching 1 or 2 oligo-DNA strand, GNP10/$(SH40b)_1$ (**1b**) or GNP10/$(SH40b)_2$ (**2b**), were mixed in complementary combinations to make specific self-organized aggregations. Figures 2 and 3 show typical results of transmittance electron microscope (TEM) observation of the aggregates. The SH40a and SH40b were fully (40 base pairs) complementary to each other, and the E85a and E85b had only 15 base pairs complementary with SH40a and SH40b, respectively. Therefore, strand exchange reactions occurred as shown in Figure 2a. In Figure 2b, 1:1 aggregates of different sizes (5 and 10 nm) of GNPs were observed. These results suggest that GNPs attaching single oligo-DNA strand (**1a** and **1b**) were successfully isolated. The population (%) of such 1:1 aggregate was about 10%. Figure 3 shows TEM observation of the mixture of **2a** + **1b**, **1a** + **2b**, and **2a** + **2b**. As expected from the combinations, different size GNP triads (large/small/large and small/large/small) were observed by mixing **2a** with **1b**, and **1a** with **2b**, respectively (Figures 3a and 3b). In the case of mixing **2a** and **2b**, linear multiple aggregates of large and small GNPs were observed (Figure 3c). The populations of these aggregates were similar with or less than **1a** + **1b** combination (10%). The

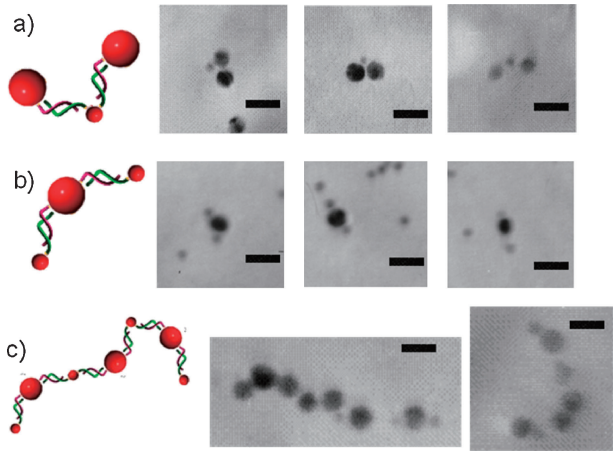


Figure 3. Schematic illustration for the GNP aggregates and TEM image of the mixture of GNP/oligo-DNA conjugates for a) **2a** + **1b**, b) **1a** + **2b**, and c) **2a** + **2b**. The scale bars are 20 nm.

reason of such low yields may be low concentration of the conjugates or detachment of SH-oligo-DNAs.

In conclusion, we could isolate GNP/oligo-DNA conjugates having defined number of oligo-DNA attached and demonstrated specific aggregation formation based on the number of oligo-DNA strands introduced and their complementary sequences. These conjugates should have potential applications as building blocks in nanotechnology and probes in biological diagnostics.

This work was financially supported by Grant-in-Aid for Scientific Research (No. 21655065) from JSPS, Japan.

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9 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

