J|A|C|S COMMUNICATIONS

Published on Web 05/15/2009

Controlling the Number and Positions of Oligonucleotides on Gold Nanoparticle Surfaces

Kenji Suzuki,* Kazuo Hosokawa, and Mizuo Maeda

Bioengineering Laboratory, RIKEN, Hirosawa 2-1, Wako, Saitama, 351-0198, Japan

Received February 13, 2009; E-mail: knjsuzuki@riken.go.jp

Nanoparticles (NPs) conjugated with oligonucleotides (ODNs) have emerged as promising materials for biological sensing as well as for bottom-up nanotechnologies based on the Watson−Crick base pairing.[1−3] Most of the previous works have been carried out using NPs having only one ODN molecule[2] or a number of ODN molecules per NP.[1] Recently, NPs with atom-like oligovalency or anisotropy have attracted much attention as building blocks for advanced nanomaterials.[4] Isolation of NPs conjugated with a known small number of ODN molecules has been established based on gel electrophoresis[4a,b] or anion-exchange HPLC.[4d] Asymmetric coating of NPs with ODNs has also been accomplished by a solid phase synthesis-like method to control the positions of ODNs on a NP.[4e] However, the number and positions of ODNs on a NP have never been controlled at the same time.

Herein, we describe a new method to immobilize a given number of ODNs on a gold nanoparticle (AuNP) in a specific arrangement directed by a geometrical template made of DNA. The basic strategy is illustrated in Figure 1. First, a set of thiolated ODNs for

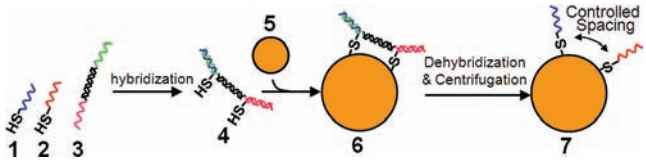

Figure 1. Schematic illustration of the strategy for controlled immobilization of ODNs onto a gold nanoparticle by utilizing a geometrical template made of DNA.

immobilization **1**, **2**, and a nonthiolated ODN template **3** are hybridized to make a DNA nanostructure **4**. Next, **4** is reacted with a AuNP **5** via the thiol groups to form a complex **6**. Finally, a AuNP/ODN conjugate **7** is obtained by removing **3** from **6**. This strategy should enable us to make various formats of AuNP/ODN conjugates simply by changing the design of the DNA nanostructure, because programmable self-assembly of DNA can generate a variety of geometries of DNA nanostructures such as triangles, squares, hexagons, and polygons.[5] In this study, we carried out proof-of-concept experiments using a linear geometry of the DNA nanostructure as illustrated in Figure 1.

First, we examined the ability of our method to control the number of immobilized ODNs per NP. The detailed experimental procedure is described in the Supporting Information (SI). Briefly, 5-, 10-, and 30-nm AuNPs were purchased from BBInternational (Cardiff, U.K.) and then coated with a phosphine ligand, bis(*p*-sulfonatophenyl)phenylphosphine dipotassium salt (BSPP), to block nonspecific adsorption of ODNs onto AuNPs.[4a] Two types of 5′-thiolated 50-mer single-stranded ODNs, HS-T_3-X-T_{17} and HS-T_3-Y-T_{17}, were used as the ODNs **1** and **2**. Each of them has its own 30-mer sequence, X or Y, between the T_3-spacer at its 5′-end and the T_{17}-tag at its 3′-end (see SI for the sequences). The addition of the T_3-spacers was to promote the reaction of the thiolated ODNs and AuNP surfaces, while the addition of the T_{17}-tag facilitated electrophoretic separation of the AuNP/ODN conjugates later. Two other types of 45-mer single-stranded ODNs, X′A′ and Y′A, were used as the nonthiolated ODNs for the template **3**. A′ and A are 15-mer sequences complementary to each other and give the double-stranded arm at the center of the template. To prepare the DNA nanostructure, these four ODNs were incubated in 10 mM Tris-HCl (pH 8) containing 100 mM NaCl at 90 °C for 10 min and then cooled down to 30 °C at a rate of −1 °C/min. The DNA nanostructure was purified by electrophoretic separation of the hybridization mixture on a polyacrylamide gel (see Figure S1). The BSPP-coated 5-nm AuNPs **5** and the purified DNA nanostructures **4** were then mixed at a molar ratio of 5:1 or 5:2 in 0.5 × TBE containing 1 mg/mL BSPP and 166 mM NaCl. The mixture was incubated at room temperature (∼22 °C) for 24 h to make **6**. Thereafter, template **3** was dehybridized from **6** in a dilute solution of BSPP (0.25 mg/mL in pure water) at 40 °C and then removed from the AuNP suspension with centrifugation at 4 °C for 1 h (at 21 600 G). For comparison, other AuNP/ODN conjugates were prepared without using the template **3** based on a published method with minor modifications.[2b,c] In this case, the BSPP-coated 5-nm AuNP and HS-T_3-X-T_{17} were mixed at a molar ratio of 5:2 or 5:4 in the same buffer solution as described above and incubated at 50 °C for 1 h. To remove unreacted ODNs, the AuNPs in the reaction mixture were washed three times with the dilute solution of BSPP.

Figure 2 shows the gel image of electrophoretic analysis of the resulting suspensions including the AuNP/ODN conjugates. Lane 1 corresponds to the 5-nm AuNPs as a negative control, while lanes 2 and 3 correspond to the AuNP/ODN conjugates made without the template. As reported previously,[4a] the suspensions of the AuNP/ODN conjugates include some different types of conjugates having one, two, and three ODNs (referred to as 1:1, 1:2, and 1:3 conjugates, respectively) in addition to unreacted AuNPs. On the other hand, our method gave a 1:2 conjugate preferentially, as shown in lanes 4 and 5. To confirm whether the 1:2 conjugate had both types of the thiolated ODNs (i.e., HS-T_3-X-T_{17} and HS-T_3-Y-T_{17}) or not, we carried out a hybridization test using two types of ODNs, T_{50}-X′ and T_{50}-Y′, as probes to the ODNs fixed on the 1:2 conjugate. In this test, the same suspensions as lane 4 were incubated with an excess amount of either of the probe ODNs or a 1:1 mixture of them for 24 h, and they were then electrophoresed in lanes 6 to 8. Clearly, the 1:2 conjugate mixed with either of the probe ODNs (see lanes 6 and 7) migrated faster than that mixed with both of the probe ODNs (see lane 8). This means that the 1:2 conjugate made with the template had both types of the thiolated ODNs. All these results strongly support the ability of our method to control the number of ODNs on a AuNP.

Next, we confirmed the ability of the proposed method to control the positions of ODNs on a AuNP using a transmission electron microscope (TEM). For easy TEM observation, 30-nm AuNPs were

10.1021/ja9011386 CCC: $40.75

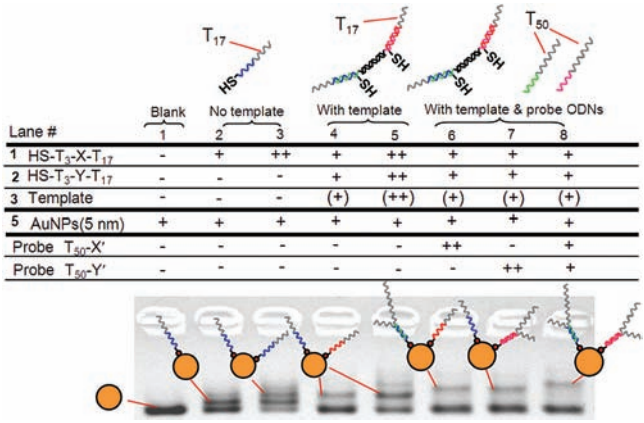

Figure 2. Electrophoretic analysis of suspensions of AuNP/ODN conjugates. Lane 1 corresponds to the BSPP-coated 5-nm AuNPs. Lanes 2 and 3 correspond to AuNP/ODN conjugates made without the template: the mixed molar ratio of AuNP to ODN was 5:2 (lane 2) or 5:4 (lane 3). Lanes 4 and 5 correspond to AuNP/ODN conjugates made by utilizing the DNA nanostructure: the mixed molar ratio of AuNP to DNA nanostructure was 5:1 (lane 4) or 5:2 (lane 5). Lanes 6 to 8 correspond to the same conjugates as those of Lane 4 after hybridization with probe ODNs: the mixed molar ratio of AuNP/T_{50}-X′/T_{50}-Y′ was 1:26:0 (lane 6), 1:0:26 (lane 7), or 1:13:13 (lane 8). The parentheses of (+) and (++) mean that template 3 was used but was afterword removed from the suspensions before the hybridization with probe ODNs.

used instead of the 5-nm AuNPs. In concert with the upsizing of the AuNPs, the 15-mer double-stranded arm composed of A′ and A in template **3** was exchanged with a 35-mer one composed of B′ and B (see SI). The DNA nanostructure **4** was made by hybridizing the ODNs for the longer template, X′B′ and Y′B, and thiolated ODNs, HS-T_3-X and HS-T_3-Y. Reaction of BSPP-coated 30-nm AuNPs and the DNA nanostructure was carried out at a lower NaCl concentration (50 mM), and then the template was removed from the complex **6** by the same dehybridization process as described above. As illustrated in Figure 3a, the positions of the immobilized ODNs on the AuNPs were visualized by hybridizing the resulting AuNP/ODN conjugate **7** with probe AuNPs **8** and **9** which had been uniformly coated with a number of ODNs complementary to **1** and **2**, respectively. The diameters of **8** and **9** were 5 or 10 nm. For comparison, other AuNP/ODN conjugates were made by using the 30-nm AuNPs without the template, and they were also hybridized with **8** and **9**. The resulting AuNP trimers **10** in these hybridization mixtures were purified based on electrophoretic separation on 2% agarose gels (see Figure S2) and observed using a TEM (see Figures S3 to S5).

Figure 3b to 3d show representative TEM images of the AuNP trimers. For each of the trimers ((b) $n = 160$, (c) $n = 136$, and (d) $n = 87$), the angle θ between the two vectors from the center of the largest AuNP to each center of the probe AuNPs were measured and summarized in the histograms. The histogram of trimers made without the template has no clear peak (see Figure 3b), whereas those of the trimers made by using the template have clear peaks between 20° and 40° (see Figure 3c and d). Based on a simple geometrical assumption (see Figure S6), the angle θ is predicted as 43°, which is in good agreement with the observed peak positions in the histograms. The broadening of the peaks may be due to the limits of the position control using the flexible DNA nanostructure having several nicks and T_3-spacers as well as due to the flexibility of the AuNP trimers. These results clearly indicate the ability of the method to control the positions of ODNs on a AuNP.

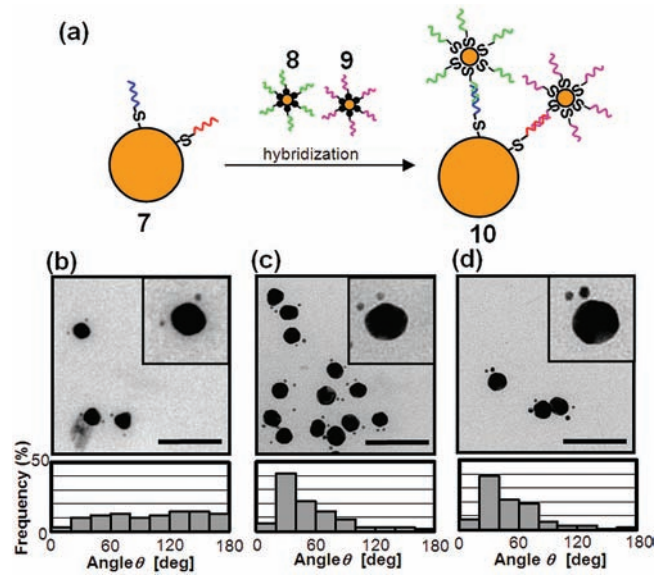

Figure 3. TEM observation of AuNP trimers. (a) Schematic illustration of the hybridization process to make a AuNP-trimer. (b) Trimers including a conjugate made without the template and 5-nm probe AuNPs. (c) Trimers including a conjugate made by using the templates and 5-nm probe AuNPs. (d) Trimers including a conjugate made by using the templates, a 5-nm probe AuNP, and a 10-nm probe AuNP. For each of the trimers in TEM micrographs, the angle between two vectors from the center of the largest AuNP to each center of smaller AuNPs was measured and summarized in the histograms below the TEM images. Scale bar = 100 nm.

In conclusion, we have developed a new method to immobilize ODNs onto a AuNP with precise control of their number and positions. This method could be extended to functionalization of NPs other than AuNPs by introducing appropriate reactive groups into ODNs to be fixed. Further studies would be needed to produce more sophisticated formats of NP/ODN conjugates using other designs of DNA nanostructures including two-dimensional or three-dimensional ones.[5]

Supporting Information Available: Experimental section, gel image data on electrophoretic purification of the AuNP trimers, and additional examples of TEM images of the AuNP trimers. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607. (b) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959. (c) Le, J. D.; Pinto, Y.; Seeman, N. C.; Musier-Forsyth, K.; Taton, T. A.; Kiehl, R. A. *Nano Lett.* **2004**, *4*, 2343. (d) Park, S. Y.; Litton-Jean, A. K. R.; Lee, B.; Weigand, S.; Schatz, G. C.; Mirkin, C. A. *Nature* **2008**, *451*, 553.

(2) (a) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez Jr, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609. (b) Loweth, C. J.; Caldwell, W. B.; Peng, X.; Alivisatos, A. P.; Schultz, P. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1808. (c) Claridge, S. A.; Goh, S. L.; Frechet, J. M. J.; Williams, S. C.; Micheel, C. M.; Alivisatos, A. P. *Chem. Mater.* **2005**, *17*, 1628. (d) Sharma, J.; Chhabra, R; Liu, Y; Ke, Y.; Yan, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 730. (e) Aldaye, F. A.; Sleiman, H. F. *J. Am. Chem. Soc.* **2007**, *129*, 4130.

(3) Niemeyer, C. M.; Bürger, W.; Peplies, J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2265.

(4) (a) Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P. *Nano Lett.* **2001**, *1*, 32. (b) Fu, A.; Micheel, C. M.; Cha, J.; Chang, H.; Yang, H.; Alivisatos, A. P. *J. Am. Chem. Soc.* **2004**, *126*, 10832. (c) Bidault, S.; de Abajo, F. J. G.; Polman, A. *J. Am. Chem. Soc.* **2008**, *130*, 2750. (d) Claridge, S. A.; Liang, H. W.; Basu, S. R.; Fréchet, J. M. J.; Alivisatos, A. P. *Nano Lett.* **2008**, *8*, 1202. (e) Xu, X.; Rosi, N. L.; Wang, Y.; Huo, F.; Mirkin, C. A. *J. Am. Chem. Soc.* **2006**, *128*, 9286.

(5) (a) Seeman, N. C. *Mol. Biothechnol.* **2007**, *37*, 246. (b) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856.

JA9011386