Triton X-100-Assisted Assembly of 5-nm Au Nanoparticles by DNA Hybridization

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This report describes a Triton X-100-assisted method to assemble small Au nanoparticles by DNA hybridization. The nonspecific adsorption of oligonucleotides, which is most prevalent on small Au nanoparticle surface and interferes with the hybridization of complementary sequences, is overcome by shielding the Au nanoparticle with a Triton X-100 layer. Amine-modified oligonucleotides, but not thiol-modified oligonucleotides, are able to penetrate the X-100 shell to enable the hybridization of complementary DNA sequences.

Mirkin's group has developed several protocols to assemble Au nanoparticles based on the molecular recognition property of complementary DNA strands.[1] These procedures work well with 15-nm particles but not with the smaller 5-nm particles. No hybridization was detected when a matching DNA linker was added to the oligonucleotide-functionalized 5-nm Au particles. Further investigations revealed the presence of strong interaction between the oligonucleotide backbone and the surface of small gold nanoparticles. The oligonucleotides in this case are recumbent on the nanoparticle surface and are therefore not optimally oriented for hybridization. The Alivisatos procedure[2] also fails to work when the number of DNA strands per Au particle is more than a few. The use of competitive MCH (6-mercapto-1-hexanol) adsorption, while successful in reducing the nonspecific adsorption of oligonucleotides on Au films,[3] could not be applied to Au nanoparticles. The recent work of Hamad–Schifferli and co-workers[4] has also pointed out that the MCH displacement of thiol-modified oligonucleotides from the Au nanoparticles (9.4 nm) is a time-dependent process, and long reaction times would result in the precipitation of the Au nanoparticles. In this work the addition of MCH to a solution of thiolated oligonucleotide-functionalized 5-nm Au particles caused the immediate precipitation of the latter, indicating that MCH not only diminishes the interaction between the nitrogen bases and the Au surface atoms, but also displaces the thiolated oligonucleotides from the Au surface. The MCH treatment is therefore unsuitable for enabling DNA hybridization on small Au nanoparticles. We have also verified that ethylene glycol monolayers, which are used to eliminate nonspecific binding of proteins on metal nanoparticles,[5] are ineffective for oligonucleotides. There is therefore a real need to overcome the problem of nonspecific adsorption on small metal nanoparticles before DNA hybridization can be accepted as a general assembly method. Herein we report a Triton X-100-assisted method that allows the assembly of small (5 nm) Au nanoparticles by DNA hybridization. In the current implementation an adsorbed Triton X-100 layer on the particle surface is used to reduce the nonspecific binding of oligonucleotides. The layer is penetratable by amine-modified oligonucleotides but not by thiol-modified oligonucleotides.

In a typical experiment 0.8 mL of 40 mM aq sodium citrate solution was added to 10 mL of 1 mM aq $HAuCl_4$ solution. Under vigorous stirring, 0.5 mL of 100 mM aq $NaBH_4$ solution was added dropwise to prepare a Au hydrosol stabilized by citrate ions. The citrate-stabilized Au nanoparticles were subsequently converted into Triton X-100-stabilized nanoparticles by adding 1 mL of 10% (w/w) aq Triton X-100 solution to the Au hydrosol and aging the mixture for several hours. TEM measurements showed a mean particle size of about 5 nm and narrow size distribution (standard deviation = 0.30 nm) for the Triton X-100-stabilized Au nanoparticles.

For the preparation of oligonucleotide functionalized small Au nanoparticles, 2 nmol of amine-modified oligonucleotides (sequence: 5′-NH_2-TTA TAT ACT TAA AAG CAA TA-3′) was added to 400 μL of the Triton X-100-stabilized Au hydrosol prepared above. The mole ratio of oligonucleotides to Au nanoparticles was about 30:1. The mixture was then aged overnight. Another mixture was likewise prepared using the same Au hydrosol and 2 nmol of amine-modified oligonucleotides of complementary sequence (5′-NH_2-TAT TGC TTT TAA GTA TAT AA-3′). 200 μL each of these oligonucleotide-modified mixtures was suspended in 0.3 M PBS buffer and then mixed together. The color of the mixed solution turned from red to purple in an hour and a precipitate was formed in ≈10 h, indicating that the Au nanoparticles were assembled by DNA hybridization.

The citrate-stabilized Au nanoparticles did not have good stability. Particle aggregation occurred immediately in 0.1 M PBS. However, the Au hydrosol after conversion to Triton X-100 protection could be stored for several hours in 0.1 M PBS, and up to several minutes in 0.3 M PBS, all without precipitation. When pristine oligonucleotides without amine modification at either ends were introduced to the Triton X-100-stabilized 5 nm Au hydrosol at a 30:1 ratio, the stability of the Au hydrosol in the PBS buffers remained unchanged. This is indication that the adsorbed X-100 layer had isolated the Au nanoparticles from the oligonucleotides. Enhanced nanoparticle stability in 0.3 M PBS, however, was observed with the use of amine-modified oligonucleotides. This is only possible if the terminal NH_2 group on the oligonucleotide could penetrate the adsorbed Triton X-100 layer to reach the underlying Au nanoparticle surface. On the contrary, the use of thiol-modified oligonucleotides did not lead to any stability enhancement, suggesting that the SH group lacks the penetrating power of the NH_2 group for the X-100-adsorbed layer. This observation is at odds with the common belief that

stronger affinity exists between Au and thiol. We currently do not have a satisfactory explanation but have observed consistently a strong affinity of the amine group for small metal nanoparitcles (Pt and Ru). The amine penetration of the X-100 functionalized Au nanoaprticles has been independently confirmed by gel electrophoresis, which showed detectable retardation in the electrophoretic mobility of the Au nanoparticles after the addition of amine modified oligonucleotides (data not shown).

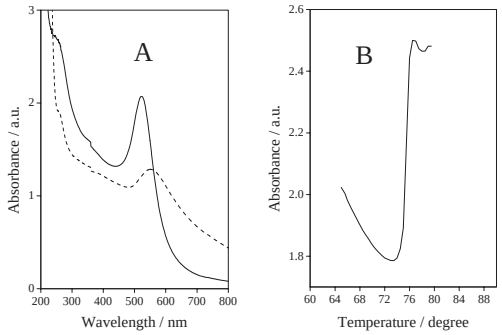

Figure 1. (A) UV–vis spectrum of 5 nm Au nanoparticles before (solid line) and after (dash line) the DNA hybridization-induced assembly. (B) "Melting temperature" analysis of the 5-nm Au assemblies induced by DNA hybridizations. The absorbance was recorded at wavelength of 260 nm for DNA solution concentration of 1 μM on a Shimadzu UV-2450 spectrophotometer with a Shimadzu DC Pico Ace 25 Peltier temperature controller. Measurements were carried out by recording the spectra at 1 °C interval with hold time of 1 min at each degree.

UV–vis spectroscopy was used to characterize the DNA hybridization-induced assembly of 5-nm Au nanoparticles. The assembly was seen as a broadening and red shifting of the Au surface plasmon band from 524 to 554 nm (Figure 1a) in the UV–vis spectrum of the Au hydrosol. Also, when the hydrosol containing aggregated Au nanoparticles was heated above the "melting temperature" (T_m) of the hybridized oligonucleotides, the initial red color of the solution and the surface plasmon band at 524 nm, characteristic of the disassembled Au nanoparticles, would reappear (Figure 1b). This "melting transition" was extremely sharp and is characteristic of the dehybridization of DNA in close proximity of metal nanoparticles.[1b,6]

The TEM image (Figure 2) of the assembled system indeed shows extended aggregates of 5-nm Au nanoparticles forming three-dimensional structures on the TEM grid. A control experiment in 0.3 M PBS buffer was carried out in which 200 μL of amine-oligonucleotide-stabilized 5-nm Au nanoparticles was mixed with 200 μL of 5-nm Au nanoparticles functionalized by amine-modified oligonucleotides with mismatched base sequences and different lengths (5′-NH_2-CGC ATT CAG GAT-3′). The experiment did not produce any observable changes in the UV–vis spectrum or the formation of aggregated structures in TEM (data not shown). Only randomly dispersed particles were detected by TEM under these conditions.

In summary, a Triton X-100-assisted method enabling the DNA hybridization induced assembly of oligonucleotide-functionalized small (5 nm) Au nanoparticles has been developed. Without the Triton X-100 treatment the oligonucleotides interact with the small Au nanoaprticles strongly and nonspecifically resulting in no apparent hybridization. Amine-modified oligonucleotides are used in lieu of the more common thiol-modified oligonucleotides because only the former are able to penetrate through the adsorbed X-100 layer. The prevalence of nonspecific interaction between metal nanoparticles of Au, Ag, Pt, and Cu and DNA has long been recognized as the bane of effective DNA hybridization.[5,7] The X-100 assisted method may provide as a solution to such problem thereby allowing more metal-DNA bioconjugates to be assembled by DNA hybridization.

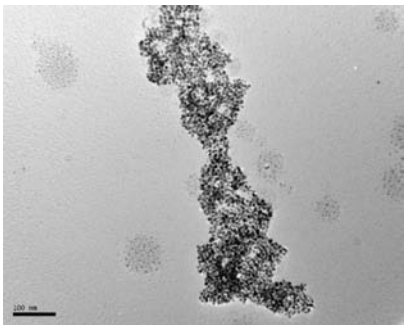

Figure 2. TEM image of 5 nm Au nanoparticle assembly induced by DNA hybridization.

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References

1 a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, and J. J. Storhoff, *Nature*, **382**, 607 (1996). b) R. C. Mucic, J. J. Storhoff, C. A. Mirkin, and R. L. Letsinger, *J. Am. Chem. Soc.*, **120**, 12674 (1998). c) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, and R. L. Letsinger, *J. Am. Chem. Soc.*, **120**, 1959 (1998). d) J. J. Storhoff, A. A. Lazarides, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, and G. C. Schatz, *J. Am. Chem. Soc.*, **122**, 4640 (2000). e) R. Jin, G. Wu, Z. Li, C. A. Mirkin, and G. C. Schatz, *J. Am. Chem. Soc.*, **125**, 1643 (2003).
2 C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos, and P. G. Schultz, *Angew. Chem., Int. Ed.*, **38**, 1808 (1999).
3 M. L. Sauthier, R. L. Carroll, C. B. Gorman, and S. Franzen, *Langmuir*, **18**, 1825 (2002).
4 S. Park, K. A. Brown, and K. Hamad-Schifferli, *Nano Lett.*, **4**, 1925 (2004).
5 M. Zheng, F. Davidson, and X. Huang, *J. Am. Chem. Soc.*, **125**, 7790 (2003).
6 Y. W. Cao, R. C. Jin, and C. A. Mirkin, *J. Am. Chem. Soc.*, **123**, 7961 (2001).
7 M. A. Hayat, "Colloid Gold: Principles, Methods, and Applications," Academic Press, New York (1989), Vol. 1, p 3.

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