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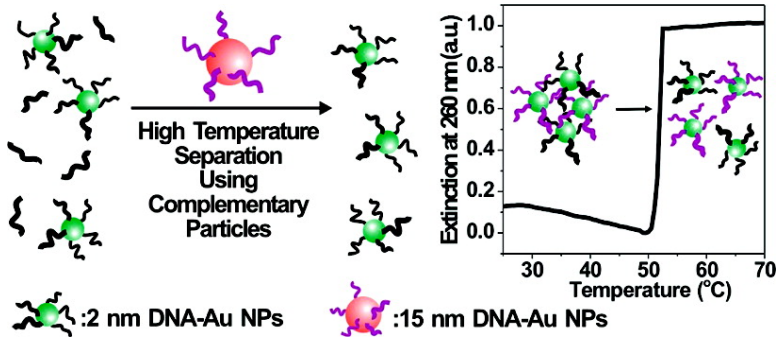

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Thermodynamically Controlled Separation of Polyvalent 2-nm Gold Nanoparticle-Oligonucleotide Conjugates

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The discovery and development of polyvalent gold nanoparticle-oligonucleotide conjugates (DNA-Au NPs) have opened a new chapter in nanobiomaterials research that is based on the unique properties of these materials.[1–3] Three of these properties include their cooperative binding, intense surface plasmons, and sharp melting transitions when hybridized to complementary nucleic acids.[1a,2a] All of these properties have been associated with relatively large diameter particle conjugates (5∼250-nm gold cores), and researchers have used such structures to develop selective materials assembly strategies,[1] highly sensitive diagnostic assays,[2] and stable cellular-transfection and gene regulation agents.[3]

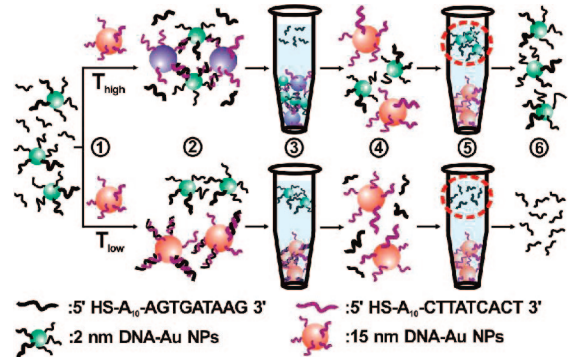
Gold nanoparticles ≤2-nm diameter represent another class of materials and have been important components of nanostructures,[4a–c] scaffolds for drug delivery,[4d] labels for electron microscopy,[4e] redox-active probes,[4f] and efficient quenchers or generators of fluorescence.[4g–i] While several groups have demonstrated that small Au NPs can be conjugated to DNA,[4g,h,5a] to the best of our knowledge, small gold nanoparticles heavily functionalized with DNA have not been isolated, characterized, or studied. These structures are difficult to isolate and separate from the excess oligonucleotides with which they are being modified.

Herein, we present a method for synthesizing 2-nm DNA-Au NPs, a simple but powerful approach for separating such particles, and a study aimed at characterizing their hybridization properties. The synthesis of these small DNA-Au NPs involves functionalization using excess thiol-DNA in the presence of salt to fully passivate the Au NP surface. Briefly, 2-nm Au NPs (Ted Pella, synthesized by reduction of gold chloride using sodium thiocyanate) were combined with thiol-terminated DNA (**DNA 1**: 5′ HS-A_{10}-AGT-GAT-AAG 3′). Salt, sodium dodecylsulfate (SDS), and phosphate were added to achieve 0.15-M NaCl, 0.01-% SDS, and 0.01-M phosphate (pH 7.4), respectively, and the solution was incubated for 12 h. During incubation, sonication was used to facilitate DNA loading on the particles.[1c] At this stage, however, a large excess of free DNA remains with the functionalized particles.

Whereas the separation of large (≥13 nm) particles relies on centrifugation, such procedures proved difficult in the case of 2-nm Au NPs. The mass of 2-nm Au NPs (≈ 4.9×10^4 g/mol) is not large enough to allow separation using normal benchtop centrifugation procedures (maximum RCF ≈ $1.6 \times 10^4 g$). In addition, these gold nanoparticles have an almost undetectable surface plasmon resonance or scattering,[6] making their characterization difficult. Moreover, approaches to separate small nanoparticles such as gel electrophoresis,[5a] diafiltration,[5b] gel filtration,[5c,d] ultracentrifugation,[5b,e] and dialysis,[5f] can be hampered by complicated instrumentation and lengthy experimental protocols.

To overcome such challenges, we hypothesized that one may be able to separate functionalized Au NPs from free, unbound, excess oligonucleotides on the basis of the difference in their binding properties. The binding constant of a DNA-Au NP for a complementary sequence has been shown to be as much as 100 times greater than a free oligonucleotide.[7] Therefore, if 2-nm DNA-Au NPs exhibit larger binding constants than free DNA, it should be possible to combine a mixture of 2-nm DNA-Au NPs and excess DNA with complementary 15-nm DNA-Au NPs at a temperature where only 2-nm DNA-Au NPs can hybridize with the 15-nm particles (Scheme 1). The resulting aggregates should then allow isolation by simple centrifugation.

Scheme 1. Schematic Illustration of the Thermodynamically Controlled Separation of 2-nm DNA-Au NPs[a]



[a] (1) Incubation with complementary 15-nm particles at a desired temperature; (2) hybridization; (3) centrifugation and removal of the supernatant; (4) redispersion in water; (5) centrifugation; (6) collection of the final product in the supernatant.

Thus, to separate the particles from the free DNA we explored their temperature dependent hybridization properties (Scheme 1). First, a mixture containing 2-nm DNA-Au NPs and excess **DNA 1** (of the same sequence) was combined with complementary 15-nm particles (**DNA 2**: 5′ HS-A_{10}-CTT-ATC-ACT 3′) at 22 °C (step 1; T_{low}). At this point, a competition exists between the 2-nm DNA-Au NPs and the free DNA for the complementary 15-nm DNA-Au NPs. However, particle aggregation does not take place under these conditions, since at low temperatures the free DNA strands hybridize with the 15-nm particles preventing hybridization with the 2-nm DNA-Au NPs (step 2; T_{low}) and subsequent aggregate assembly. We next conducted the same competition experiment (steps 1, 2) at an elevated temperature (T_{high}, 30 °C, vide infra). In this case, aggregation was observed, as indicated by a red-to-purple color change (see Supporting Information). This color change is a diagnostic indication of the 2-nm DNA-Au NPs and their ability to cross-link with the complementary 15-nm DNA-Au NPs to form aggregate structures. The mixture was centrifuged, and the supernatant was removed (step 3). The precipitates were collected, washed, and redispersed in water to dehybridize all duplex links (step 4). The 15-nm DNA-Au NPs were separated by centrifugation (step 5), and the supernatant containing the isolated product was collected (step 6).

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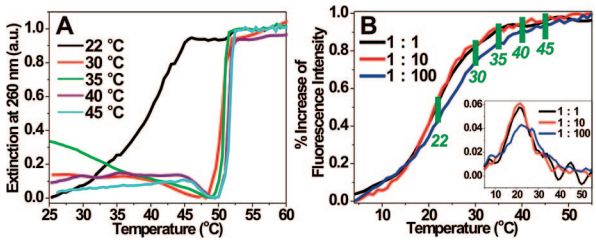

Figure 1. (A) Melting profiles of hybridized products separated at 22, 30, 35, 40, and 45 °C and collected after step 6 (Scheme 1). (B) Melting profiles of 15 nm Au NPs hybridized with the complementary fluorescein-labeled DNA (at a 1:1, 1:10, and 1:100 ratio), and their first derivatives (inset). The separation temperatures used (Figure 1A) are noted in Figure 1B.

To verify that the products isolated using T_{high} conditions were 2-nm DNA-Au NPs and probe their binding properties, we combined two sets of complementary 2-nm particles and measured their melting transition using UV–vis spectroscopy. Interestingly, the hybridized particle mixture displayed a sharp melting transition at 51.5 °C (Figure 1A, 30 °C). Importantly, the narrow breadth of the melting transition (full width at half-maximum (fwhm) of the first derivative = 1.3 °C) is indicative of a particle with a dense loading of DNA capable of cooperatively binding to similar particles with complementary sequences.[1a–c] This type of transition is observed even after repeated melting and subsequent hybridization processes (see Supporting Information).

A solution containing separated 2-nm DNA-Au NPs was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) and a DNA fluorescence quantification assay to determine the concentrations of gold and DNA, respectively (see Supporting Information). Consistent with assumption of dense oligonucleotide loading, the surface coverage of the 2-nm DNA-Au NP is 64.8 ± 6.4 pmol/cm^2 (~5 strands per particle), which is significantly higher than larger DNA-Au NPs (14~19 pmol/cm^2).[1c] Electrophoresis was used to confirm that, within the limits of the technique, the separated particles are free of excess DNA (see Supporting Information). The overall yield was 46%.

We next investigated the thermodynamic control afforded by the separation procedure that leads to the isolation of densely functionalized 2-nm DNA-Au NPs. The separation protocol was conducted at 22, 30, 35, 40, and 45 °C, and the hybridization and subsequent melting properties of the separated products were examined. Products from the 22 °C separations exhibit a very broad transition (fwhm = 8.5 °C, Figure 1A, 22 °C), indicating that they are primarily free DNA. Products from high temperature separations ($T \geq 30$ °C), however, exhibit very sharp transitions (fwhm = ~1.5 °C, Figure 1A, 30–45 °C), indicating that they are primarily 2-nm DNA-Au NPs.

To further investigate the properties that enable the separation process, we conducted melting experiments using 15-nm Au NPs hybridized with complementary fluorophore-labeled oligonucleotides (**F-oligo**: 5′ fluorescein-T-AGT-GAT-AAG 3′). The ratio of 15-nm DNA-Au NPs to **F-oligo** was 1:1, 1:10, or 1:100 to simulate the possible concentrations of free DNA remaining after synthesis. Regardless of the concentration of **F-oligo**, all of the solutions exhibit broad melting transitions (fwhm = 9.4 °C) with a melting temperature (T_m) at 22 °C (Figure 1B). Therefore, when the separation procedure is performed at 22 °C (the midpoint of the melting transitions) a significant amount of free DNA can still hybridize to the 15-nm DNA-Au NPs, and the product contains this free DNA, as evidenced by a broad melting transition (Figure 1A, 22 °C). At 30 °C or higher, however, hybridization of free DNA is not likely (Figure 1B). Therefore, primarily 2-nm Au NP-DNA conjugates bind to 15-nm particles at these elevated temperatures (T_m = 52.1 °C, see Supporting Information). These experiments confirm that the separation process is afforded by the increased T_ms and cooperative properties of 2-nm DNA-NPs.

In conclusion, we have developed a novel method to isolate densely DNA-functionalized 2-nm Au NPs, and discovered that these materials exhibit increased T_ms and cooperative properties. Because of these properties, we can easily separate 2-nm DNA-Au NPs from free oligonucleotides on the basis of thermodynamic control. It should be possible to use this approach to separate other densely DNA-functionalized materials such as quantum dots,[5f] virus particles,[8] or dendrimers,[9] thus overcoming many of the challenges commonly associated with handling and manipulating nanomaterials not suitable for conventional separation procedures. Moreover, the discovery that small DNA-Au NPs exhibit cooperative binding properties should motivate researchers to investigate and utilize these classes of materials for a variety of assembly, diagnostic, and therapeutic applications.

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Supporting Information Available: Materials, experimental procedures, and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643–1654. (b) Lee, J.-S.; Stoeva, S. I.; Mirkin, C. A. *J. Am. Chem. Soc.* **2006**, *128*, 8899–8903. (c) Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313–8318. (d) Dilenback, L. M.; Goodrich, G. P.; Keating, C. D. *Nano Lett.* **2006**, *6*, 16–23. (e) Niemeyer, C. M.; Simon, U. *Eur. J. Inorg. Chem.* **2005**, 3641–3655.

(2) (a) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609. (b) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078–1081. (c) Nam, J. -M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**, *301*, 1884–1886. (d) Lee, J.-S.; Han, M. S.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4093–4096. (e) Cerruti, M. G.; Sauthier, M.; Leonard, D.; Liu, D.; Duscher, G.; Feldheim, D. L.; Franzen, S. *Anal. Chem.* **2006**, *78*, 3282–3288. (f) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547–1562. (g) He, L.; Musick, M. D.; Nicewarner, S. R.; Salinas, F. G.; Benkovic, S. J.; Natan, M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 9071–9077. (h) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643. (i) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768–11769.

(3) (a) Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. *Science* **2006**, *312*, 1027–1030. (b) Seferos, D. S.; Giljohann, D. A.; Rosi, N. L.; Mirkin, C. A. *ChemBioChem* **2007**, *8*, 1230–1232.

(4) (a) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609–611. (b) Andres, R. P.; Bein, T.; Dorogi, M.; Feng, S.; Henderson, J. I.; Kubiak, C. P.; Mahoney, W.; Osifchin, R. G.; Reifenberger, R. *Science* **1996**, *272*, 1323–1325. (c) Zubarev, E. R.; Xu, J.; Sayyad, A.; Gibson, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 15098–15099. (d) Gibson, J. D.; Khanal, B. P.; Zubarev, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 11653–11661. (e) Birrell, G. B.; Hedberg, K. K.; Griffith, O. H. *J. Histochem. Cystochem.* **1987**, *35*, 843–853. (f) Templeton, A. C.; Cliffel, D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1999**, *121*, 7081–7089. (g) Maxwell, D. J.; Taylor, J. R.; Nie, S. *J. Am. Chem. Soc.* **2002**, *124*, 9606–9612. (h) Dubertret, B.; Calame, M.; Libchaber, A. J. *Nat. Biotechnol.* **2001**, *19*, 365–370. (i) Huang, C.-C.; Yang, Z.; Lee, K.-H.; Chang, H.-T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6824–6828.

(5) (a) Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P *Nano Lett.* **2001**, *1*, 32–35. (b) Sweeney, S. F.; Woehrle, G. H.; Hutchison, J. E. *J. Am. Chem. Soc.* **2006**, *128*, 3190–3197. (c) Woehrle, G. H.; Brown, L. O.; Hutchison, J. E. *J. Am. Chem. Soc.* **2005**, *127*, 2172–2183. (d) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. *J. Chem. Soc., Chem. Commun.* **1994**, 801–802. (e) Kanaras, A. G.; Kamounah, F. S.; Schaumburg, K.; Kiely, C. J.; Brust, M. *Chem. Commun.* **2002**, 2294–2295. (f) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1999**, *121*, 8122–8123.

(6) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17–30.

(7) Lytton-Jean, A. K. R.; Mirkin, C. A. *J. Am. Chem. Soc.* **2005**, *127*, 12754–12755.

(8) Strable, E.; Johnson, J. E.; Finn, M. G. *Nano Lett.* **2004**, *4*, 1385–1389.

(9) Choi, Y.; Mecke, A.; Orr, B. G.; Banaszak Holl, M. M.; Baker, J. R., Jr. *Nano Lett.* **2004**, *4*, 391–397.

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