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Improving the Yield of Mono-DNA-Functionalized Gold Nanoparticles through Dual Steric Hindrance

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Supporting Information

ABSTRACT: A novel strategy of dual steric hindrance, which was obtained by Janus modification of gold nanoparticles (Au NPs) and volume exclusion of DNA, was adopted to prepare mono-DNA-modified Au NPs. The yield of mono-DNA-functionalized Au NPs significantly improved from 44 to 70% in the reaction between Au NPs and thiolated DNA. Furthermore, the specificity of mono-DNA-functionalized Au NPs was enhanced from 57 to 95%. The as-prepared Au NPs without postsynthetic treatment showed good controllability in self-assembly fabrication of complex nanostructures.

DNA-based programmable assembly of gold nanoparticles (Au NPs) into well-defined superstructures has been attracting much attention because of the high designability, good structural control, and potential applications in plasmonics and electronics.[1,2] One of the significant challenges in DNA−Au NP hybrid superstructures is controlling the number of DNA on the surface of the modified Au NP building blocks (e.g., to have each Au NP conjugated with only one DNA chain). These mono-DNA-modified Au NPs are critical for the fabrication and subsequent application of geometry- and structure-controlled assemblies.[2a,3−5] Current strategies to obtain mono-DNA-modified Au NPs are mainly based on selective separation of them from the mixed products of Au NPs modified with different numbers of DNA using gel electrophoresis[6] or anion-exchange HPLC,[7] and the yield is typically no more than 30%.[8,9] Such postsynthesis treatments are time- and cost-consuming, preventing practical applications of DNA−Au NP superstructures. Therefore, it is imperative to develop simple methods to produce mono-DNA-modified Au NPs with high throughput and specificity. In this work, by adopting a new strategy based on dual steric hindrance (i.e., Janus surface modification of Au NPs and volume exclusion of triofliumrepens DNA molecules), we demonstrate remarkable improvements in the yield and specificity of mono-DNA-modified Au NPs in the reaction of Au NPs and DNA. As-prepared Au NPs without postsynthetic treatment could be used to construct assemblies with complex and controlled structures.

Figure 1 outlines the experimental process for preparing mono-DNA-functionalized Au NPs through steric hindrance. First of all, the Janus Au NPs were synthesized by a modification of the reported procedure.[10] As shown in step a in Figure 1, negatively charged Au NPs would spontaneously adsorb onto the surface of positively charged silica colloids because of electrostatic attraction when they were mixed in solution. Transmission electron microscopy (TEM) images [Figure 2a and Figure S1 in the Supporting Information (SI)] and dynamic light scattering (DLS) data (Figure S2) confirmed that the bis(*p*-sulfonatophenyl)phenylphosphine (BSPP)-stabilized Au NPs with average diameters of 3.5 nm were homogenously dispersed and tightly attached to the surface of silica colloids with a mean size of 80 nm. Subsequently, Au NP/silica colloid composites were centrifuged and redispersed in a solution containing glutathione (Glu). Because of the strong interaction between Au and the mercapto group of Glu, the BSPP stabilizers on the surfaces of Au NPs exposed to the solution were replaced by Glu molecules to form Janus NPs (step b in Figure 1; also see Figure S3). Janus Au NPs were finally obtained by HF etching to eliminate the electrostatic attraction between Au NPs and silica colloids, removal of the silica colloids from the solution, and redissolution in water. The TEM images (Figure 2b) and electrophoresis results (Figure 2c and lanes 9 and 18 in Figure 3e) indicated that the Janus Au NPs had a similar diameter (∼3.5 nm), size distribution (∼10%), and surface charge (both BSPP and Glu have net negative charges in tris-borate/EDTA buffer solution) in comparison to the original ones.

Subsequently, triofliumrepens DNA molecules were added to the solution of Janus Au NPs (step d in Figure 1). Because the interaction between the Au NP and the phosphor group of BSPP is much weaker than that between Au and the mercapto group of DNA, triofliumrepens DNA molecules preferentially replaced BSPP stabilizers on the Janus Au NP surface (red part in Figure 3a). Furthermore, the diameter of triofliumrepens DNA molecules in the extended configuration was ∼4 nm, which is comparable to the size of the Au NPs. Because of the large volume exclusion effect, attachment of two or more triofliumrepens DNA molecules on the same side of a Janus Au NP was highly unfavorable energetically, so it was expected that mono-DNA-modified Au NPs would be obtained in high yield (Figure 3a). Analysis of the electrophoresis results confirmed the effect of steric hindrance (Figure 3e). When the mixing ratio of Janus Au NPs to triofliumrepens DNA was 1:3, the yield of

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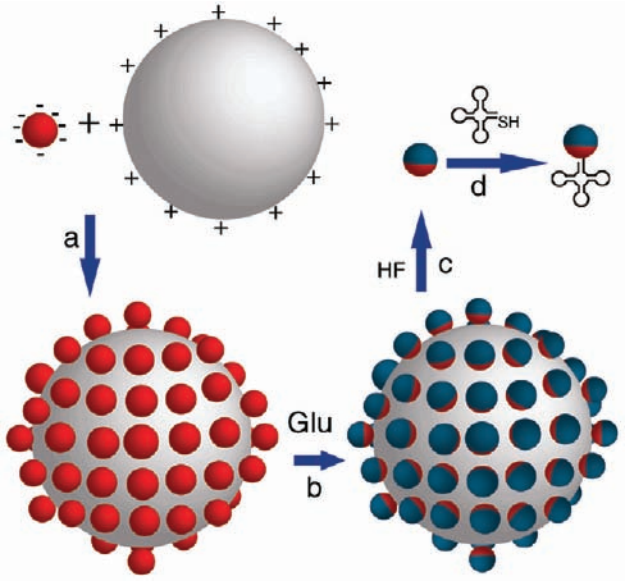

Figure 1. Strategy for conjugation of a single DNA onto a Au NP through dual steric hindrance. Red spheres stand for homogeneous Au NPs and gray ones for silica colloids. The red and blue parts of the Au NPs represent the BSPP and Glu stabilizers on the Au NPs, respectively.

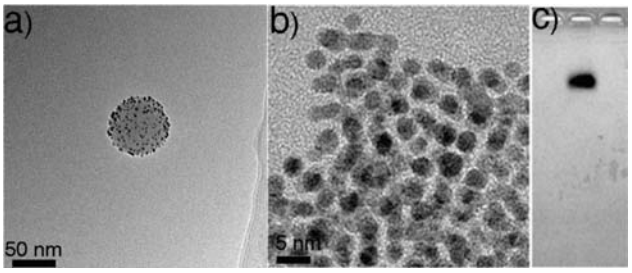

Figure 2. (a, b) TEM images of (a) the composite of 3.5 nm Au NPs and 80 nm silica colloids and (b) Janus Au NPs after removal of the silica colloids. (c) Electrophoresis picture of Janus Au NPs.

mono-DNA-modified Au NPs could be up to 70% (lane 1 in Figure 3e; the detailed calculation is described in the SI). The other 30% consisted of unreacted Janus Au NPs (27%) and Janus Au NPs modified with two or more DNA (3%). The specificity of mono-DNA-modified Au NPs (S_{mono}) is defined as follows:

$$S_{mono} = \frac{Y_{mono}}{Y_{mono} + Y_{multi}}$$

where Y_{mono} is the yield of mono-DNA-modified Au NPs (70%) and Y_{multi} is the yield of Au NPs modified with two or more DNA (3%). Therefore, we calculate the specificity of mono-DNA-modified Au NPs to be ~95%. Such a high specificity will remarkably benefit the further application of DNA-modified Au NPs, because the unreacted Au NPs cannot interact with the complementary DNA (cDNA) and therefore have a negligible effect.

Serial control experiments were carried out to highlight the importance of the steric hindrance effect on the production of mono-DNA-modified Au NPs (Figure 3b–d). As the steric effect in our strategy is mainly produced through volume exclusion by DNA and Janus modification of the Au NPs simultaneously, a

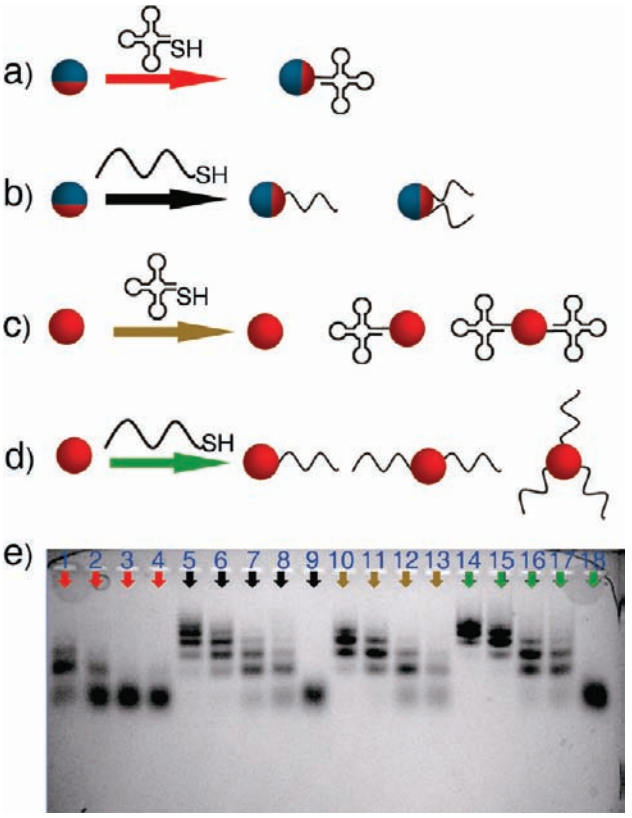

Figure 3. (a–d) Scheme and (e) electrophoresis results for conjugation of Au NPs with DNA at different ratios. (a) Steric hindrance induced by both the Janus morphology and triofliumrepens DNA [lanes 1–4 in (e)]. (b) Steric hindrance solely from the Janus morphology (lanes 5–9). (c) Steric hindrance caused only by triofliumrepens DNA (lanes 10–13); (d) Mixture of homogeneous Au NPs and linear DNA (lanes 14–18). The red and blue parts in (a–d) represent the BSPP and Glu stabilizers on the Au NPs respectively. The red, black, gold, and green arrows in (e) stand for the different reactions in (a–d), respectively. The Au NPs/DNA ratios in lanes 1–18 in (e) are 1:3, 1:2, 1:1, 2:1, 1:3, 1:2, 1:1, 2:1, 1:0, 1:3, 1:2, 1:1, 2:1, 1:3, 1:2, 1:1, 2:1, and 1:0, respectively. The concentration of NaCl was kept at 18.75 mM.

single or no steric hindrance effect was investigated. First, when Janus Au NPs were mixed with linear DNA in different ratios (Figure 3b and lanes 5–8 in Figure 3e), the highest yield and specificity of mono-DNA-modified Au NPs were ~52% and 61%, respectively (lane 7 in Figure 3e). These values are much lower than those obtained using Janus Au NPs and triofliumrepens DNA, suggesting that the secondary structure of the DNA remarkably enhances the yield and specificity of the mono-DNA-modified Au NPs. This result is easily understood in terms of the fact that compared with triofliumrepens DNA having an extended structure, linear DNA molecules encounter a much weaker repulsion force when they are conjugated onto the same NP. Second, in order to evaluate the effect of Janus modification of Au NPs on the yield and specificity of mono-DNA-modified Au NPs, homogeneous Au NPs were used instead of Janus-modified Au NPs. When reactants were homogeneous Au NPs (without Janus modification of Glu) and triofliumrepens DNA at different ratios (Figure 3c and lanes 10–13 in Figure 3e), the highest yield and specificity of mono-DNA-modified Au NPs were ~53% and 76%, respectively. This result demonstrates that the Janus structure effectively reduces the active part of Au NPs

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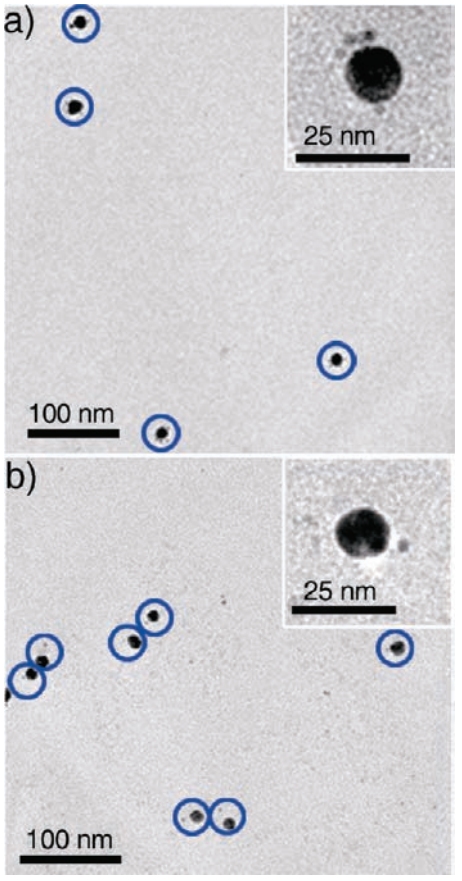

Figure 4. TEM images of satellite structures formed by monotriofliumrepens DNA-modified Janus Au NPs (3.5 nm) and cDNA-modified Au NPs (13 nm) in ratios of (a) 25 and (b) 2.5. The insets in (a) and (b) show magnified images of satellite structures.

and thus improves the yield and specificity of mono-DNA-modified Au NPs. Finally, when neither Janus surface modification of Au NPs nor volume exclusion of triofliumrepens DNA was adopted (Figure 3d and lanes 14−17 in Figure 3e), the highest yield and specificity of mono-DNA-modified Au NPs were only 44% and 57% (lane 17 in Figure 3e), respectively. These experimental results demonstrate that the more steric effects are used, the larger is the improvement in the yield and specificity of mono-DNA-modified Au NPs. The corresponding statistical results for each of the lanes in Figure 3e are summarized in Table S1 in the SI.

Finally, in order to confirm that Au NPs bearing a single triofliumrepens DNA could be functionalized with other DNA sequences, we fabricated satellite structures by assembling as-prepared mono-DNA-modified Au NPs (without further purification) with 13 nm Au NPs bearing multiple cDNA (Figures S4−S9 and Figure 4). Two prominent assembly features were recognized: (1) Separated satellite structures were obtained in high yield, showing not only successful assembly with cDNA-modified Au NPs but also elimination of cross-talk between the satellite structures. Previous studies indicated that complementary assembly of NPs modified with multiple DNA molecules easily leads to large and disordered aggregation, because each NP can provide multiple binding sites to the NPs modified with the cDNA.[11,12] On the contrary, every mono-DNA-modified Au NP could interact with only one Au NP bearing cDNA,[13] so separated satellite structures were prepared controllably. (2) The assembly units in the satellite structures might be controlled by simply altering the mixing ratios of DNA-modified Au NPs. For instance, when the ratio of 3.5 nm Au NPs to 13 nm Au NPs was 25, >70% of the satellite structures contained 2−5 3.5 nm Au NPs (Figure 4a and Figure S5). As the ratio of 3.5 nm Au NPs to 13 nm Au NPs was changed to 2.5, ~80% of the satellite structures contained just one 3.5 nm Au NP (Figure 4b and Figure S6). The statistical results are shown in Figure S7. Without the existence of triofliumrepens DNA on Janus Au NPs, corresponding satellite structures were hardly observed (Figure S8). The above experimental observations suggest that monotriofliumrepens DNA-modified Au NPs will be good candidates for the design and manipulation of the structures of NP assemblies.

In conclusion, we have developed a high-throughput method for the production of mono-DNA-modified Au NPs that takes advantage of dual steric hindrance caused by Janus surface modification of Au NPs and volume exclusion by triofliumrepens DNA. The resultant Au NPs show the capability of further assembly with other DNA-modified nanomaterials in a controllable way. Moreover, this strategy can be used to connect single DNA molecules with other types of NPs such as quantum dots or magnetic NPs,[14−16] which will open the door to the fabrication of DNA-based functional nanostructures.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental methods, DNA sequences, statistical results for electrophoresis lanes, and satellite structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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