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Research Letter

Assembly/Disassembly of DNA-Au Nanoparticles: A Strategy of Intervention

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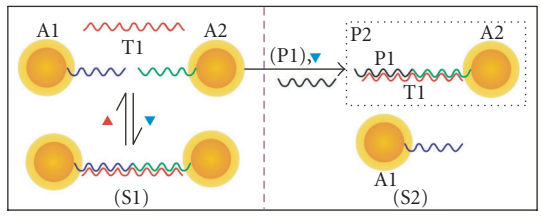
This report describes the viability of a strategy for manipulating the assembly/disassembly processes of DNA-Au nanoparticles by molecular intervention. Using the temperature-induced assembly and disassembly processes of DNAs and gold nanoparticles as a model system, the introduction of a molecular recognition probe is demonstrated to lead to the intervention of the assembly/disassembly processes depending on its specific biorecognition. This process can be detected by monitoring the change in the optical properties of gold nanoparticles and their DNA assemblies. Implications of the preliminary results to exploration of the resulting nanostructures for fine-tuning of the interfacial reactivities in DNA-based bioassays and biomaterial engineering are also discussed.

1. INTRODUCTION

The pioneering work on DNA mediated assemblies of gold nanoparticles by Mirkin et al. [1–3] has opened the door to a host of potential applications in biological sensing, medical diagnostics, and drug delivery. Extensive research efforts have aimed at understanding the interactions and reactivities involved in the DNA-based nanoparticle assembly [4–26]. Examples include DNA-gold nanoparticles with fluorescence signatures [7], sequence-dependent stability of DNA-modified gold nanoparticles [8], quenching of single-stranded DNA-linked nanoparticles [12, 13], a DNA hybridization assay using barcoded metal nanowires [14–17], single-base mismatched associated fluorescent quenching [18], SERS-based multiplexed detection [19], grazing-angle-FTIR probing of orientations in DNA-nanoparticle hybridization [20], improvement of the sensitivity and photostability of DNA-hybridizations using dye-doped nanoparticles [21], and restriction enzyme disassembly and DNA ligase reassembly of a DNA-nanoparticle network [22, 23]. While much has been learned about the assembly process, relatively little is known about the disassembly process. The ability to control both assembly and disassembly using biorecognition capabilities of DNA or proteins in combination with nanoparticles is important in biological processes, especially in drug delivery, gene therapy, immunotherapy, and in a wide range of biological probes and sensors.

In this report, we describe the preliminary results of a study of the viability of manipulating the assembly/disassembly processes of DNA and Au nanoparticles by molecular intervention. We were not the first in studying the assembly/disassembly processes. The temperature-induced assembly and disassembly processes of DNAs with gold nanoparticles demonstrated by Mirkin et al. [1–3] served as a model system for demonstrating the proof-of-concept of our intervention strategy. As conceptually illustrated in Scheme 1, the introduction of a molecular recognition probe (P1) into a solution of two types of DNA-anchored nanoparticles, for example, DNA1-capped Au (A1) and DNA2-capped Au (A2) in the presence of a target DNA (T1) as reported previously [1–3], leads to the possibility of the intervention of the assembly/disassembly processes depending on its recognition with A1, A2, or T1. For example, if recognition occurs between P1 and T1, the presence of P1 could prevent A1 from reassembling with T1 as the solution temperature is reduced. This process can be detected by monitoring the change in the optical properties of gold nanoparticles and their DNA assemblies. Thus, the formation of P2 provides



Scheme 1: A schematic diagram (not to scale) illustrating: (S1) the assembly and disassembly of DNA1-capped Au (A1) and DNA2-capped Au (A2) nanoparticles via a target DNA (T1) upon changing the temperature (▲ = heat to 75–80°C; ▼ = gradually cooling down to 25°C (RT)); and (S2) an intervention of the disassembly by the introduction of P1 into the heated solution which leads to the formation of P2 upon returning to room temperature.

opportunities for further tuning of the interfacial reactivities in the DNA-nanoparticle bioassay or biomaterial engineering, which constitutes the basic motivation of this work.

The temperature-dependent assembly/disassembly processes (S1) have been documented [1–3]. To demonstrate the viability of the intervention process (S2), we used the same oligonucleotides as used previously [1–3], which include DNA1: 5′-TCTCAACTCGTA/3ThioMC3-D/3′, DNA2: 5′/5ThioMC6-D/CGCATTCAGGAT-3′, and DNA3: 5′-TACGAGTTGAGAATCCTGAATGCG-3′ (Integrated DNA Technologies, Inc.) with standard desalting purification and citrate-capped gold nanoparticles (Au_{nm} 11.4 ± 0.8 nm) which were synthesized using the reported procedure [27]. The DNA1 and DNA2 were first dissolved in 0.1 M phosphate buffer (pH 8) at a concentration ranging from 300 to 370 μM. The disulfide bonds in DNA1 and DNA2 were cleaved using an approach similar to the reported procedure [1–3] where dithiothreitol (DTT) was added at 0.1 M final concentration to ~10 OD of the nucleotides in a final volume of 400 μL. The solution was allowed to react at room temperature for 2 hours, then put through a NAP-5 column (Amersham Biosciences), and an aliquot of 1.1 mL phosphate buffer (pH 8) was added to the column to elute the cleaved oligonucleotide. The final concentration of the cleaved DNAs was 20 μM with an $OD_{260\,nm}$ of 2.2. The exact concentrations of DNAs varied slightly depending on the specific experiment.

The surface of gold nanoparticles was functionalized with the cleaved 5′ and 3′-thiol modified oligonucleotides [1–3] to form A1 and A2. Briefly, 1 mL of the cleaved DNAs (1 or 2) separately was added to 5 mL of gold nanoparticles (stock concentration 14 nM). The solution was left standing at room temperature for 16 hours after which it was diluted to 0.1 M NaCl and 10 mM phosphate buffer (pH 7) and allowed to stand for another 40 hours at room temperature. The DNA-capped nanoparticles were then centrifuged and washed twice at 14000 rpm for 25 minutes (each time the solution was redispersed in a 0.1 M NaCl/10 mM phosphate buffer (pH 7) solution) before being redispersed in its final (0.3 M NaCl/10 mM phosphate buffer (pH 7) and 0.01% sodium azide) solution and stored at room temperature. In some cases, the solution of DNA-capped nanoparticles was heated up to 50°C for 10 minutes before centrifugation. Depending on the batch of nanoparticles and DNA used, the concentration of each component varied slightly.

The gold nanoparticles used in this study display a surface plasmon (SP) resonance band at about 520 nm (Figure 1), the shift of which is correlated to the change in the size, interparticle distance, and dielectric medium [28–32]. As shown in Figure 1(a), the solution of the DNA capped nanoparticles (A1 + A2) (red solution) exhibited an SP band at 525 nm. Upon the addition of T1 in room temperature, the SP band shifted gradually from 525 nm to ~560 nm within a 30-minute time frame, after which a decrease in the absorbance began to occur as a result of the precipitation of the assembly solution due to the formation of large clusters. The color of the solution changed from red to purple during this process. TEM images for samples from the A1 or A2 solution showed scattered and isolated nanoparticles, whereas the sample containing the A1, A2, and the T1 DNA exhibited highly-clustered features of the nanoparticle assemblies (left panel of Figure 1(a)). The degree of clustering is dependent on the concentration of the nanoparticles, oligonucleotides, and salts, in addition to temperature (data not shown). This assembly/disassembly process is reversible as demonstrated by the spectral evolution in the process of heating the solution above the DNA's melting temperature and the process of returning to room temperature (Figure 1(b)) [1–11]. In this example, the solution was heated to 80°C for 5 minutes, where the solution displayed a red color and the SP band shifted to 525 nm. As the solution temperature was cooled down, the color of the solution turned purple which is accompanied by the redshift and the broadening of the SP band. These observations are consistent with those reported previously [1–11].

As an example, we used uncleaved DNA1 (5′-TCTCAACTCGTA/3ThioMC3-D/-3′) as P1 to demonstrate the viability of process-S2 in Scheme 1. When the solution of the DNA-nanoparticle assembly was heated to 80°C, a controlled amount of P1 (12X the T1 DNA concentration) was added to the solution. The solution temperature was kept at 65°C for additional 5 minutes (Figure 1(c)). When the solution was cooled down to room temperature, the SP band remained at 525 nm without any detectable broadening toward longer wavelengths. The color remained reddish. This observation is in sharp contrast to the observation for the same process for A1 + A2 + T1 in the absence of P1 (Figure 1(b)).

The contrast in the spectral evolution is indicative of the intervention of P1 in the assembly process, which served as an example demonstrating the viability of manipulating the complementary binding of DNAs in the assembly/disassembly processes. The absence of a change in the solution color and the spectral absorbance for this second process suggested that P1 has taken the place of A1 in terms of the complementary binding, which can be explained by the difference in size and mobility between P1 and A1. In comparison with A1 which has DNA1 attached onto the nanoparticle surface, P1 has a smaller size and a faster mobility. Therefore, the reactivity between P1 and T1 upon cooling

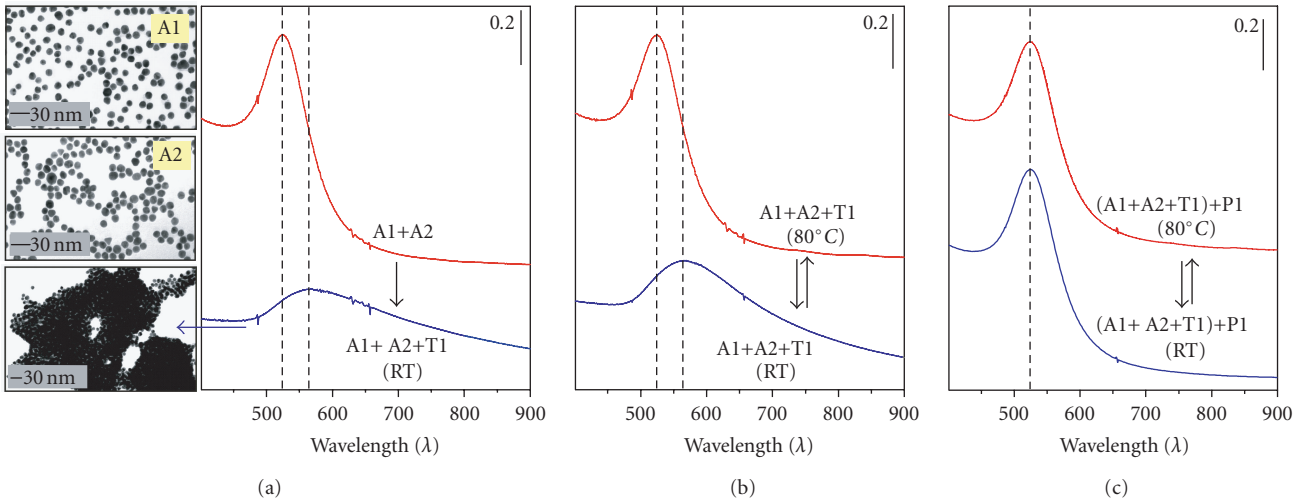
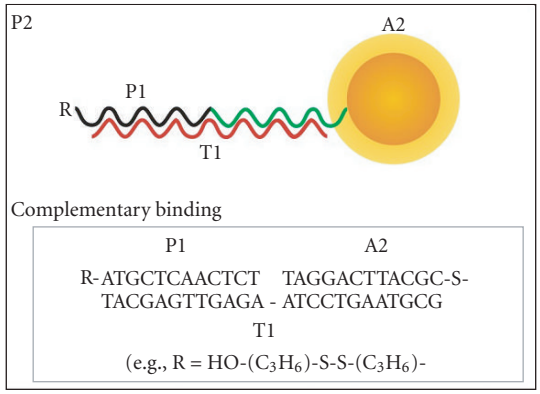


FIGURE 1: Spectral evolution of the SP band showing the assembly of DNA capped nanoparticles (A1 and A2) in the presence of a target DNA (T1) (a), the reactivity upon heating and cooling of solution-A (b), and the reactivity upon heating and cooling of solution-A in the presence of an uncleaved DNA (P1) (c). TEM images of samples from A1, A2, and the assemblies are included in the left panel for comparison. (Note that the scale bar for the assemblies is smaller in order to show the degree of clustering.) Concentration for various components: $[Au_{nm}] = 3.3$ nM; [DNA1 or DNA2] = 1.0 μM; [T1] = 0.1 μM; [P1] = 1.2 μM.



SCHEME 2: Illustration of the complementary binding (not to scale) in the structure of P2, and the detailed binding of A2 and P1 in the presence of its complementary DNA (T1).

the temperature is favored over A1 in terms of the complementary binding to T1, as illustrated in Scheme 2. Based on the concentrations of A1 or A2 (after centrifugation), T1, and P1 used in the reaction and as a spherical model for Au nanoparticles, the estimated surface coverage of the thiolate-oligonucleotides, under optimized surface packing conditions, was about 150 per particle [7] with ~25% being bound to T1. The amount of P1 added to the solution (~350 P1 molecules per nanoparticle and 12 times the T1 concentration) is therefore more than sufficient to hybridize to all T1 moieties before they interact with A1. It is expected that the proportion of intervention should be dependent on the relative concentration of P1 versus the other constituents in the solution.

While the preliminary results call for further investigation into the detailed structures and reactivities of P2, the demonstration of the viability of the intervention strategy for manipulating the assembly/disassembly processes of DNA and nanoparticles potentially opens opportunities for expanding this strategy for applications in biorecognition-based assay and biomaterials engineering. For example, the disulfide moieties on the outmost shell structure of P2 provide the binding sites for attachment to different types of nanoparticles in terms of size and functionality. The cleavage of the disulfide bonds can introduce free thiol ligands for immobilization on different nanoparticles or substrates. The tailoring of the functional properties of the R-ligand could potentially serve as probes in fine-tuning the interfacial reactivity. Depending on the identity or reactivity of the R group, the surface chemistry of the DNA-anchored nanoparticles can be tailored to meet specific technological applications. These and other related possibilities such as enzymatic disassembly and DNA ligase reassembly are subjects of our on-going in-depth investigations. More detailed quantitative work on the dependence of the reactivity on the concentrations of the DNAs involved and the optimization will be reported in the near future.

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