

DNA-directed Seeded Synthesis of Gold Nanoparticles without Changing DNA Sequence

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Abstracts

DNA has been used for directing the growth of noble metal nanoparticles into different morphologies. Most previous studies focused on the effect of DNA sequence, while the effect of DNA adsorption was not thoroughly explored. In this work, we controlled the seeded growth of AuNPs by using the same DNA sequence but under different initial adsorption conditions: room temperature and heating. DNA adsorbed by heating induced more anisotropic nanoparticle growth, and the most effect was observed with 100 nM C30 DNA, where nanoflowers were obtained for the heated sample. By measuring DNA adsorption and desorption, heating did not increase DNA adsorption density but increased the adsorption affinity. The percentage of adsorbed DNA before the growth was only about 10%, regardless of heating, while after the growth, the associated DNA reached 75% or more, indicating that the free DNA also influenced the growth. This study offers fundamental insights into the effect of DNA adsorption on seeded AuNP growth, providing a method to tune the morphology of nanoparticles without changing DNA sequence.

Introduction

Using DNA for controlling the growth of noble metal nanoparticles has been carried out by many research groups, and different DNA sequences and lengths resulted in various morphologies.^[1] For example, poly-A30 or poly-C30 DNA can grow gold nanosphere seeds into nanoflowers,^[2] whereas T30, G20, C30, and A30 mediated morphological evolution of gold nanoprism seeds into nonagon, hexagon, and six-pointed stars.^[3] The morphology of gold nanorods can be directed into dumbbell-shaped, octahedral, and cracked octahedral structures in the presence of A20, G20, and T20/C20.^[4] DNA can also be used for the morphological control of spherical Ag nanoparticles to form nanoprisms, nanoflowers, and nanodiscs.^[5] In addition, bimetallic nanoparticles such as Au-Ag, Au-Pt, Au-Pd, Au-Pd-Au were also synthesized by DNA-mediated growth.^[6] The color change of these nanoparticles during the growth has been coupled with the molecular recognition properties of DNA to design biosensors.^[7] In addition, such hybrid nanomaterials have been used for drug and DNA delivery applications.^[8]

A typical synthesis starts with incubation of a DNA sequence with gold nanoseeds. Then HAuCl_4 and a reducing agent are added to initiate the growth.^[9] The DNA is believed to first adsorb on the nanoseeds to function. In all previous work, DNA was adsorbed by simple incubation.^[8a, 10] Both DNA and typical AuNP seeds are negatively charged and adsorption is unfavorable by simple mixing.^[11] DNA adsorption to AuNPs has been extensively studied, and it can be accelerated by various conditions,^[12] especially by adding salt and acid.^[13] In addition, heating and freezing were also reported to promote the adsorption of DNA.^[14] However, none of these strategies were tested for the seeded growth.

DNA adsorption is a time-dependent slow process,^[15] but the effect of DNA adsorption kinetics has yet to be examined for the seeded growth of AuNPs. We hypothesized that it might be possible to control the seeded growth of AuNPs by the same DNA sequence but under different initial adsorption conditions. In this study, we used heating to affect DNA adsorption since no additional reagents were needed, and the solution property remained the same. We have dissected the effects of the adsorbed DNA and the free DNA in solution, offering new insights and more controls into DNA-mediated growth of AuNPs.

Results and Discussion

Heating affects DNA adsorption and growth. Among various methods to promote DNA adsorption,^[12a, 16] we chose to use heating since no additional reagents were needed. We respectively adsorbed four types of 30-mer DNAs (A30, T30, C30, and G30) on AuNS seeds under two conditions: (1) room temperature (RT), and (2) heated in boiling water for 3 min followed by cooling to room temperature (Heated). Then, NH_2OH and HAuCl_4 were added to both samples to grow the seeds. Depending on the state of DNA adsorption, the resulting AuNPs might have different morphologies (Figure 1A).

At RT, the AuNPs synthesized with different DNA sequences showed different colors and surface plasmon resonance (SPR) peaks attributable to different affinities of DNA to AuNSs and their differential interactions with Au^{3+} ions (Figure 1B-G), which was consistent with the literature.^[9] Comparing the RT and Heated samples, no obvious color or SPR peak difference was observed in the absence of DNA (Figure 1B), or in the presence of A30 (Figure 1C) or G30 (Figure 1F). In contrast, the solution colors differed for T30 (Figure 1D) and C30 (Figure 1E). When the surface plasmon peak wavelengths were compared, C30 had the largest shift of 44 nm between the RT and Heated groups ($p < 0.001$, Figure 1G). Note that the seeded growth reaction would always happen regardless of DNA, where a blue/purple color indicates anisotropic growth and red color indicates isotropic enlarge of the spherical seeds.^[17]

Heating can change the adsorption of DNA to AuNSs (affinity and/or density). We speculated that A30 and G30 had a strong adsorption affinity and the seed surfaces were already favorably adsorbed by these DNA even at the RT condition, and so heating had little further effects. This conclusion was supported by the blue color of these two samples, and consistent with the previous literature.^[2] For T30, its adsorption ability was too weak.^[18] Although heating might have influenced T30 adsorption, the effect on the growth was still quite weak. Poly-T DNA is known to be less effective in mediating the growth of AuNSs.^[19] For C30, heating had the largest effect. The pH of the solution was 6.2 before adding NH_2OH , 4.6 after adding NH_2OH and 3.0 after adding HAuCl_4 . Poly-C DNA is known to form i-motif structure under acidic pH, which would disfavor the adsorption. We characterized the i-motif using the intrinsic fluorescence of DNA, where the fluorescence of C30 significantly enhanced under the synthesis condition (Figure S1).^[20] Heating can disrupt or melt such secondary structures to promote DNA adsorption, which can explain the unique effect of C30.

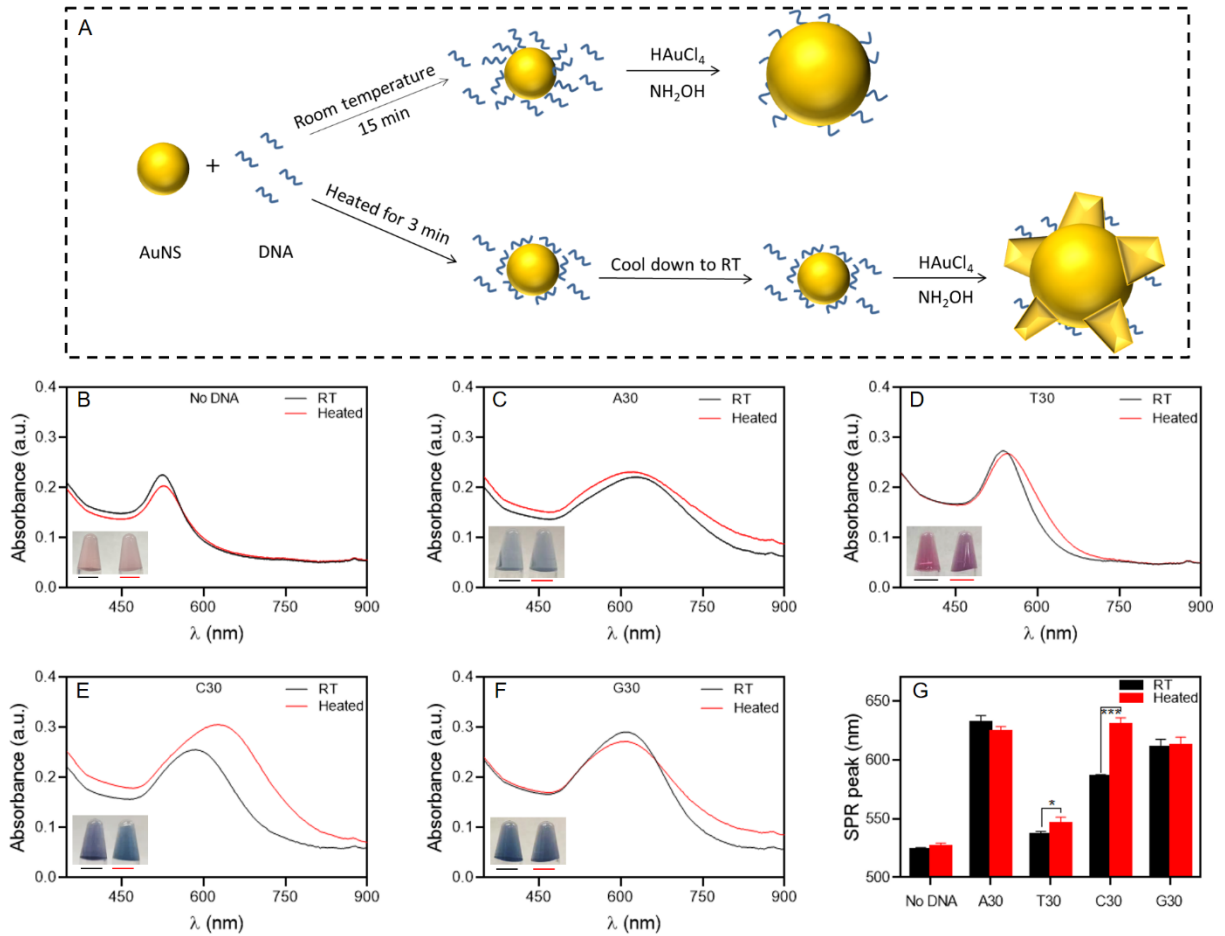


Figure 1. (A) Schematic illustration of seeded AuNP growth with DNA adsorbed at the RT or Heated conditions. By using different DNA adsorption conditions, the resulting AuNPs might be different. UV-vis spectra of the AuNPs prepared (B) without DNA, with 1 μ M (C) A30, (D) T30, (E) C30, or (F) G30 DNA at the RT or Heated conditions. Inset: photographs of the corresponding AuNPs. (G) Surface plasmon peak wavelengths of the corresponding AuNPs. * $p < 0.05$, *** $p < 0.001$.

Effect of DNA concentration. In the above experiments, the concentration of DNA was 1 μ M, which was typically used by most researchers.^[2, 21] We then varied the concentration of DNA from 10 nM to 1 μ M to optimize DNA concentration. Interestingly, at low concentration of C30 of 10 nM (Figure 2A), heating induced little change of the solution color or the SRP peak. This may be attributed to at low concentrations of C30, few DNA strands were on the surface regardless of heating. With 100 nM C30 (Figure 2B), the color change was very obviously, and

the SRR peak red-shifted from 536 nm to 622 nm (Figure 2F). This change was even greater than that with 1 μ M C30 (Figure 2C, Figure 2F). Thus, heating made the most difference in the intermediate concentration of the DNA.

The concentration of the AuNS seeds was 0.5 nM, and when 1 μ M DNA was added, the DNA-to-AuNS ratio reached 2000:1. Under typical conditions, each 13 nm AuNS can only adsorb around 20-30 nonthiolated DNA oligonucleotides,^[22] and thus the majority of the DNA (~99%) remained free in solution, regardless of heating. Therefore, with a high DNA concentration, the effect of heating should be related to the adsorbed DNA instead of the free DNA.

We also tested different concentrations of A30/T30/G30 (Figure 2D, E, G, Figure S2), and little SRR peak red-shift or solution color difference was observed between the RT and Heated samples. Overall, the sequence of DNA played a decisive role in the nanoparticle growth, and DNA concentration and heating during adsorption can further influence it.

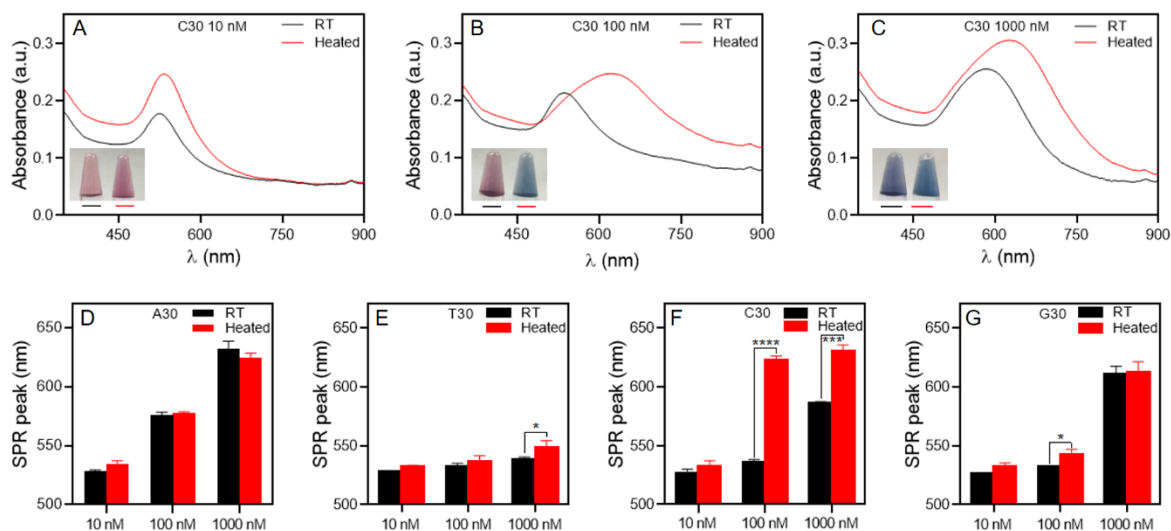


Figure 2. UV-vis spectra of AuNPs prepared with the addition of (A) 10 nM, (B) 100 nM, and (C) 1000 nM C30 under the RT and Heated conditions. Insets: photographs of corresponding AuNPs with the addition of C30 in different temperature. SPR peak wavelengths of corresponding AuNPs with the addition of different concentrations of (D) A30, (E) T30, (F) C30, or (G) G30 under the RT and Heated conditions. * p<0.05, *** p<0.001, **** p<0.0001.

Effect of temperature and heating time. To further investigate the heating effect, we adsorbed 100 nM C30 on the AuNS seeds, heated the samples in boiling water for different times, and then performed the seeded growth. As the heating time increased, the SPR peak red shifted more with the solution color turned from purple to blue (Figure 3A, B), indicating that the a longer heating time promoted anisotropic growth of AuNPs. We also varied the incubation temperature from 25°C to 100°C (Figure 3C and 3D), and found little growth change below 60°C. As the temperature further increased, a significant color change was achieved, suggesting that below 60°C, the thermal energy was insufficient to help DNA overcome the energy barrier.

To study the morphology of the AuNPs grown with C30 DNA, transmission electron microscopy (TEM) was employed (Figure 3E and 3F). The AuNSs were 13 nm spheres (Figure S3A), the nanoparticles prepared at the RT condition were enlarged spheres, whereas the nanoparticles prepared under the Heated condition grew into a nanoflower-like shape, indicating heating can induce more anisotropic nanoparticles growth. At a lower temperature of 80°C, the anisotropic growth was less obvious (Figure S3B).

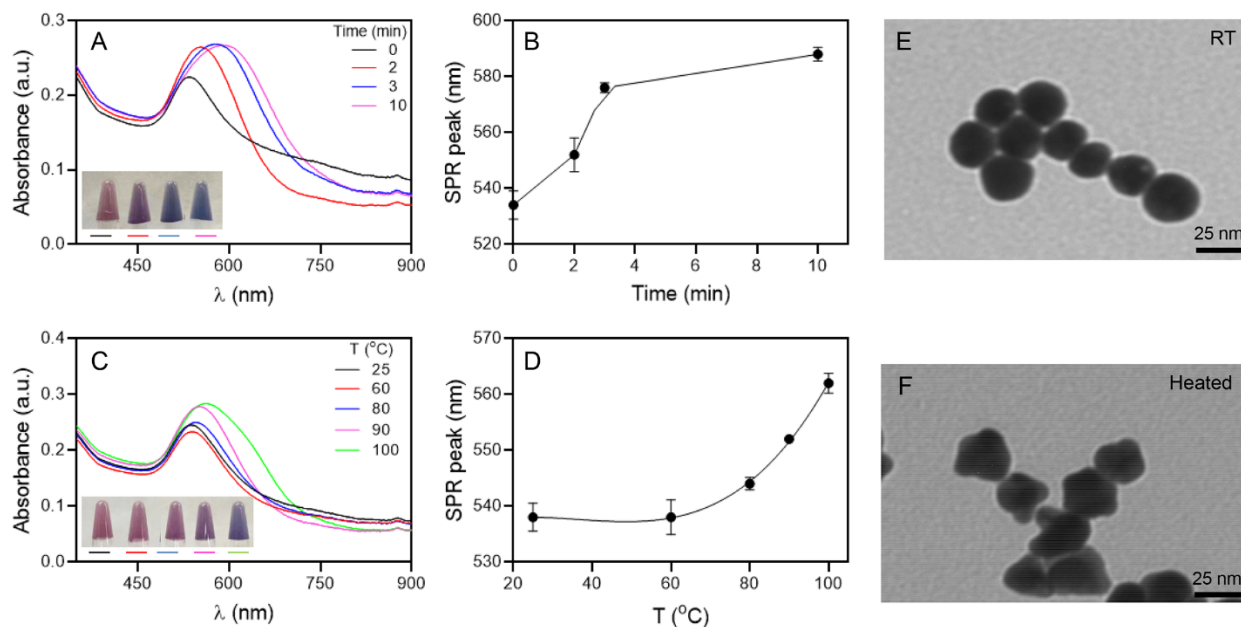


Figure 3. (A) UV-vis spectra of the AuNPs grown with the addition of 100 nM C30 with different heating times. Inset: photographs of the corresponding AuNPs grown with C30 with different heating times. (B) SPR peak wavelengths of the corresponding AuNPs in (A). (C) UV-vis spectra of the AuNPs grown with the addition of 100 nM C30 with different heating

temperatures. Inset: photographs of the corresponding AuNPs grown with C30 with different heating temperatures. (D) SPR peak wavelengths of the corresponding AuNPs in (C). TEM micrographs of the AuNPs grown with 100 nM C30 under the (E) RT and (F) Heated conditions.

Effect of heating on DNA adsorption. The above work indicated that heating might affect the adsorption of C30, which in turn affected the seeded growth. Upon heating, DNA adsorption density and affinity might be affected.^[2] To understand the mechanism behind the effect of heating, FAM (carboxyfluorescein)-labeled C30 (1 μ M) was respectively adsorbed on AuNSs (5 nM) at the RT and Heated conditions. After centrifugation and removal of non-adsorbed DNA, KCN was added to release the FAM-C30 in the pellet. We also measured the fluorescence emission spectrum of the supernatant, from which we calculated the percentage of DNA adsorption. As shown in Figure 4A, both groups had around 10% of adsorbed DNA, and thus 90% of the DNA remained in solution. Therefore, the effect of heating was not to achieve a higher DNA density, since the DNA density difference was quite small.

We then probed the DNA adsorption affinity by adding 0.5 mM KCN to the re-dispersed pellets. With such a low concentration of KCN, the AuNPs were not dissolved and we only probed desorption of FAM-C30 DNA. For both samples, about 25% DNA desorbed, but desorption was faster for the RT group with a rate constant (k) of $2.4 \pm 0.2 \text{ min}^{-1}$, while the Heated group had a k of $0.56 \pm 0.01 \text{ min}^{-1}$. This suggested that the DNA adsorbed via heating had a stronger adsorption affinity. Likely, the DNA tended to adsorb lengthwise with more contacting points to the AuNP surface after heating. This is reasonable since DNA adsorption is largely a kinetically controlled process due to the very strong DNA base adsorption.^[11] Heating allowed breaking of the initial i-motif structure of C30 and also more energy to adjust the conformation of DNA on AuNPs to achieve more stable adsorption. We depicted this process in Figure 4C. Heating promoted DNA adsorption was previously reported.^[16] In this work, we showed its application in seeded growth of AuNPs. Finally, when 20 mM KCN was added, all the remaining ~75% DNA desorbed for the two groups (Figure 4B).

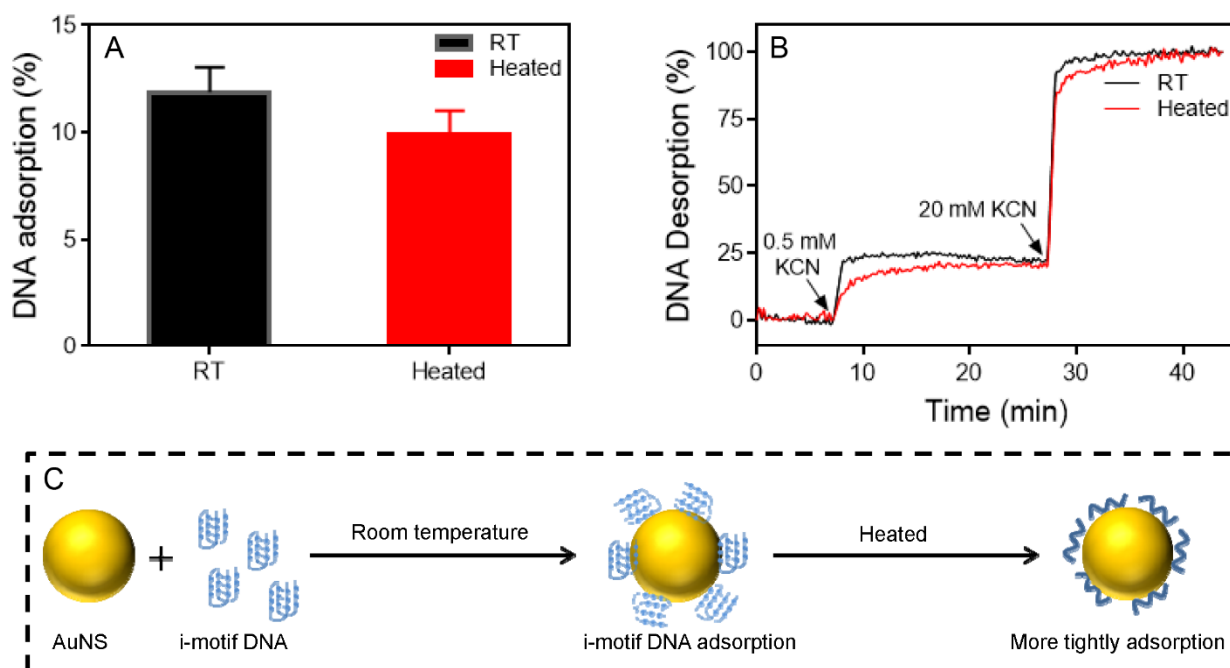


Figure 4. (A) DNA adsorption on the AuNSs under RT or Heated conditions. (B) Desorption kinetics of FAM-C30 from AuNSs indicated by the fluorescent enhancement upon the addition of 0.5 mM and 20 mM KCN. (C) Schematic Illustration of C30 DNA adsorption on AuNSs at RT and Heated conditions. Heating can disrupt its i-motif structure to result in a different adsorption state, which in turn changed the growth of the AuNPs.

Effect of removing non-adsorbed DNA. Since the majority of the DNA was not adsorbed, the next question is whether these free DNA contributed to the seeded growth or not. To investigate the influence of the free C30 DNA, they were removed after centrifugation (Figure 5A). For the AuNPs grown at RT, the solution appeared to be pink with the SPR peak blue-shifted indicating less anisotropic growth. Therefore, the free DNA also participated in the anisotropic growth of AuNPs. After adding A30 and C30 back to the samples, the nanoparticle solutions became purple after the growth, while adding T30 still resulted in red color (Figure 5B, C). This confirmed that the free DNA in solution can influence the growth of AuNPs. In the Heated samples, the trend was the same (Figure 5D, E), but the SPR peak red-shifted more compared with the RT group. Therefore, both DNA adsorbed on the AuNSs and free in solution can influence the seeded growth.

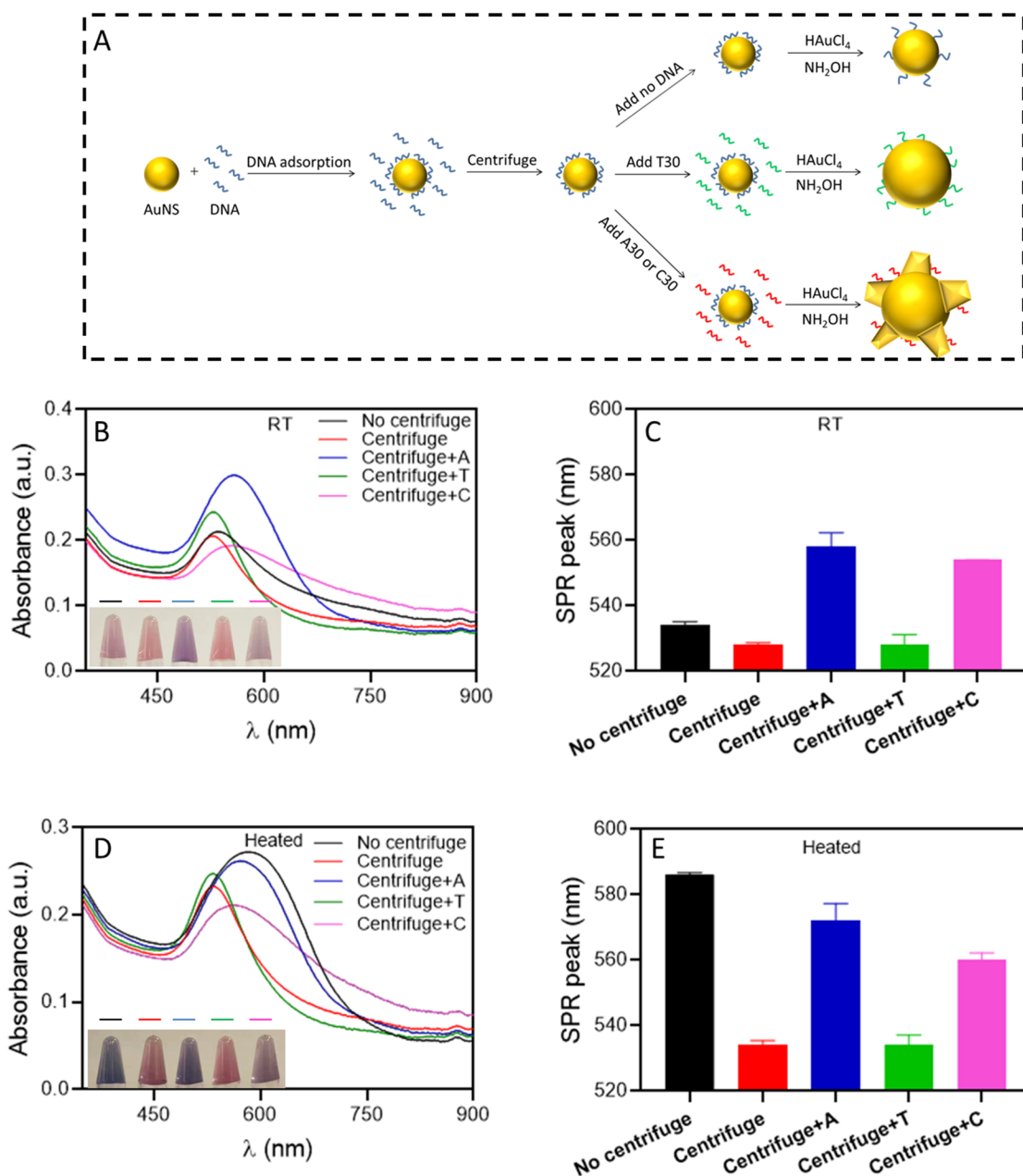


Figure 5. (A) Schematic illustration of AuNPs prepared with the adsorption of C30, and then removing the free C30 DNA in solution and finally adding different DNA sequences for the growth. UV-vis spectra of the AuNPs prepared with AuNSs incubated with C30 under the (B) RT and (D) Heated conditions, then removing the supernatant C30 and finally adding different DNA for the growth. Inset: photographs of the corresponding AuNPs. (C) and (E) The SPR peak wavelengths of the corresponding AuNPs in (B) and (D), respectively.

Incorporation of free DNA during growth. Since the free DNA contributed to the seeded growth, we then measured whether the free DNA was incorporated into the final AuNPs during the growth. 1 μM C30 was first adsorbed on 5 nM AuNSs under the RT or Heated conditions. After centrifugation, the precipitate was then redispersed in water and 1 μM FAM-labeled C30 was added, followed by adding NH_2OH and HAuCl_4 to induce the growth. We then measured the fraction of DNA adsorption, and compared the results before centrifugation (already presented in Figure 4A). As shown in Figure 6A, the DNA adsorption reached $> 75\%$ under RT and Heated growth conditions, which was much higher than that adsorbed DNA before the growth (about 10%), indicating the free DNA participated the particle growth by adsorbing on the newly formed particle surface and the DNA was incorporated in the form AuNP structure.

We then added different concentrations of KCN to desorb the DNA. Figure 6B shows that after adding 0.5 mM KCN, the DNA desorption rate were the same under the RT and Heated conditions ($k=0.14\pm0.01 \text{ min}^{-1}$), which was lower than that of adsorbed DNA before seeded growth ($2.4\pm0.2 \text{ min}^{-1}$ for RT and $0.56\pm0.01 \text{ min}^{-1}$ for Heated), indicating the DNA was more strongly associated to the AuNPs after the seeded growth. These were newly adsorbed DNA, and thus the RT and Heated conditions did not affect their adsorption affinities since the heating step was only applied to the initially adsorbed DNA before the growth. So, most of the free DNA were adsorbed on the newly formed AuNPs during the growth and this was how the free DNA contributed to the particle growth.

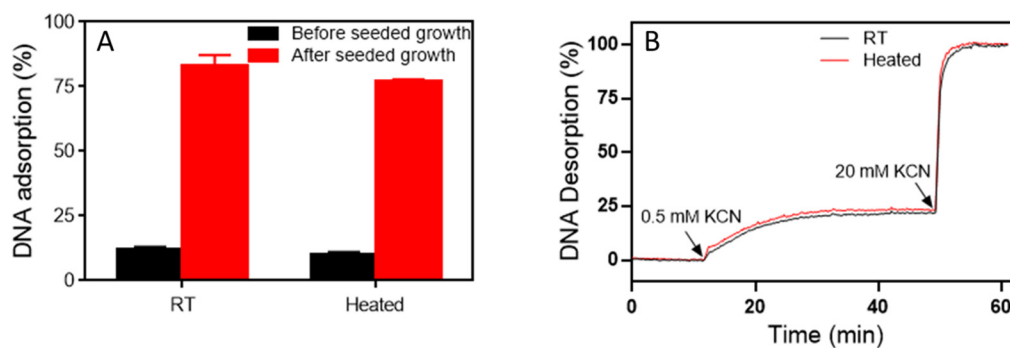


Figure 6. (A) FAM-labeled C30 DNA adsorption on the AuNSs before (black bars) and after (red bars) the growth of the AuNSs under RT or Heated conditions. (B) Desorption kinetics of FAM-C30 from AuNSs indicated by the fluorescent enhancement upon the addition of 0.5 mM and 20 mM KCN.

Conclusions

In this work, we controlled the seeded growth of AuNS by the same DNA sequence but under different initial adsorption conditions. The biggest difference between the RT and Heated conditions was seen for C30 DNA, which was attributed to its i-motif structure at acidic pH of the synthesis condition. The AuNPs prepared with C30 at RT were nanospheres but under grew into nanoflowers the Heated condition, indicating heating can influence DNA adsorption, which in turn affected nanoparticle growth. The heating time and temperature also have influence on the nanoparticle growth by affecting DNA adsorption affinity. Only about 10% DNA was adsorbed on AuNS when DNA was incubated with AuNS, while the majority of the DNA were in solution. During the growth, the free DNA participated by adsorbing on the newly formed gold surfaces and the final DNA adsorption surpassed 75%. We verified that the free DNA in solution also played an important role in the nanoparticle growth. Thus, both the adsorbed DNA and the free DNA in solution influenced the growth, and heating can change the final morphology of nanoparticles by affecting the initial adsorbed DNA. This finding provides deep insights into the DNA-mediated the nanoparticle growth and offers new ways to tune the morphology of nanoparticles for sensing,^[23] drug delivery,^[24] and catalysis.^[25]

Experimental Section

Chemicals

All of the DNA samples were purchased from Integrated DNA Technologies (Coralville, IA, Table S1). Potassium cyanide (KCN), hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) and gold (III) chloride trihydrate ($\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$) were from Sigma-Aldrich. Trisodium citrate dihydrate, sodium chloride (NaCl), sodium hydroxide (NaOH), sodium phosphate monobasic monohydrate, and sodium phosphate dibasic heptahydrate were obtained from Mandel Scientific (Guelph, ON, Canada). Gold nanospheres (AuNSs, 13 nm) were synthesized according to the literature using citrate reduction.^[26] Milli-Q water was used to prepare the buffers and solutions.

DNA-directed synthesis of AuNPs

The concentration of the 13 nm AuNSs was calculated based on Beer–Lambert law (extinction coefficient at 520 nm: $9.4\times 10^8 \text{ M}^{-1}\text{cm}^{-1}$) and then diluted adjusted to 0.5 nM in water. 150 μL of 0.5 nM AuNS solution was first incubated with 10 nM, 100 nM or 1 μM of DNA under two

conditions: (1) room temperature (RT) for 15 min, or (2) heated at 100°C for 3 min and then cooled to room temperature (Heated). After that, 7.5 μ L of 0.4 M NH_2OH (pH 5) and 1.58 μ L of 16.7 mM HAuCl_4 were then added to the AuNSs and reacted for 15 min.

DNA adsorption on AuNSs

To measure DNA adsorption, 150 μ L of 5 nM AuNSs were incubated with 1 μ M of FAM-C30 (FAM: carboxyfluorescein) under the RT or Heated conditions. The fluorescence of FAM-C30 DNA can significantly increase upon heating and the process is irreversible.^[27] To avoid such an influence, FAM-C30 was first heated at 95°C for 1 min and cooled down to room temperature before incubation with the AuNSs. After incubation, the solutions were centrifuged at 14000 rpm for 15 min at room temperature. The precipitate was then redispersed in 150 μ L of Milli-Q water. Then, 5 μ L supernatant was mixed with 95 μ L 10 mM phosphate buffer (PB) pH 7.6 for fluorescence measurement. In addition, 5 μ L of the redispersed precipitate was added to 95 μ L of 10 mM PB, pH 7.6 containing 20 mM KCN to dissolve the AuNSs and fully release the adsorbed DNA. The fluorescence emission was measured (Ex: 490 nm; Em: 530 nm, Varian Eclipse). For DNA desorption kinetics, 10 μ L re-dispersed precipitate was mixed with 90 μ L of 10 mM PB, pH 7.6 in the cuvette. The initial fluorescence (F_0) was first monitored for 5 min (Ex: 490 nm; Em: 530 nm). KCN (0.5 mM final concentration) was then added into the cuvette to release the adsorbed FAM-labeled DNA. After the signal reached saturation, KCN (20 mM final concentration) was added to dissolve the AuNSs and fully release the remaining FAM-labeled DNA.

AuNP synthesis after removing supernatant DNA

150 μ L of 0.5 nM AuNSs were incubated with 100 nM C30 under the RT or Heated conditions followed by centrifugation (14000 rpm, 15 min, 25°C). The precipitate was then redispersed in 150 μ L of Milli-Q water. Afterwards, 100 nM of DNA (A30, T30, or C30) was added. Finally, NH_2OH and HAuCl_4 were then added to induce the reaction.

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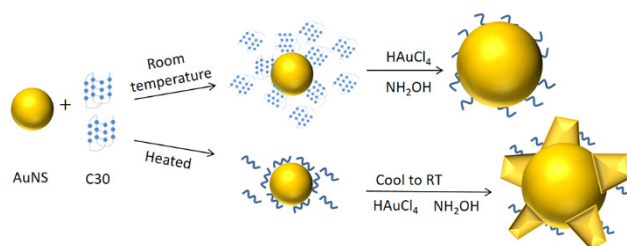
Keywords: gold nanoparticles; aptamers; biosensors; adsorption; oligonucleotides

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Graphical abstract



354
 355 Seeded growth of gold nanoparticles by the same DNA sequence but under different initial adsorption
 356 conditions yielded different products. Heating induce more anisotropic nanoparticle growth. The effects
 357 of the adsorbed DNA and the free DNA in solution were dissected, offering new insights into DNA-
 358 mediated growth.