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Spectroscopic studies of nucleic acid additions during seed-mediated growth of gold nanoparticles

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Abstract

The effect of adding nucleic acids to gold seeds during the growth stage of either nanospheres or nanorods was investigated using UV-Vis spectroscopy to reveal any oligonucleotide base or structure-specific effects on nanoparticle growth kinetics or plasmonic signatures. Spectral data indicate that the presence of DNA duplexes during seed ageing drastically accelerated nanosphere growth while the addition of single-stranded polyadenine at any point during seed ageing induces nanosphere aggregation. For seeds added to a gold nanorod growth solution, single-stranded polythymine induces a modest blue-shift in the longitudinal peak wavelength. Moreover, a particular sequence comprised of 50% thymine bases was found to induce a faster, more dramatic blue-shift in the longitudinal peak wavelength compared to any of the homopolymer incubation cases. Monomeric forms of the nucleic acids, however, do not yield discernable spectral differences in any of the gold suspensions studied.

Keywords

Au; biomimetic; colloid

INTRODUCTION

Gold nanoparticles (AuNPs) have been heavily investigated for their unique size- and shape-dependent optical properties. These effects are apparent in the variation of optical spectra that gold nanoparticles exhibit for different sizes and shapes ranging from spheres (plasmon

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Supporting Information Available; TEM micrographs and additional UV-Vis spectra of nanoparticle suspensions (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

band at ~520 nm) to nanorods (transverse and longitudinal plasmon bands at ~520 and 600–1600 nm, respectively).^{1–3} These signature spectral bands correspond to a localized surface plasmon resonance effect caused by the coherent, collective oscillation of conduction band electrons in AuNPs illuminated with light.¹ In addition to the effects of size and shape on the resulting absorption and scattering of light, changes in the spatial arrangement of AuNPs can shift the position of the plasmon bands.^{4–9} AuNP surfaces can be conveniently modified to incorporate other moieties such as oligonucleotides, proteins and antibodies due to the strong binding interaction between gold and chemical moieties such as thiols.^{7, 10–13} As a result of these practical optical properties and conjugation possibilities, gold nanoparticles have been studied in biosensing, molecular imaging, therapeutic and medical diagnostic applications.^{6, 12, 14}

Synthesis of gold nanoparticles of various shapes is typically performed via reduction of Au(III) ions in an aqueous solution with a capping agent present to stabilize formed particles. Common capping agents include citrate or cetyltrimethylammonium bromide (CTAB).^{2, 11, 12, 15–18} While synthesis protocols can vary^{19, 20} once gold seeds have nucleated, gold nanospheres can be formed by simply allowing the solution to sufficiently age.²¹ To form gold nanorods (AuNR), on the other hand, gold seeds are typically added to a growth solution containing silver nitrate to promote preferential growth of the {111} facets on the ends of the gold nanorod.^{22–24} The aspect ratio of the AuNR can be controlled through choices in growth solution conditions such as changing the concentration of silver nitrate.^{2, 15, 16, 25}

Over the past several years, numerous studies have focused on using grafted oligonucleotides to induce recognition-based assembly of gold nanoparticles as highlighted in recent reviews.^{26–29} The studies typically conjugate single-stranded thiol-modified, complementary oligonucleotides on one or more populations of nanoparticles and then induce nanoparticle aggregation as hybridized duplexes bridge nanoparticle surfaces.^{30–32} As opposed to employing DNA as a nanoparticle assembly tool, select studies have explored the use of oligonucleotides and nucleotides as templates for the synthesis of inorganic nanoparticles. Similar to prior work using peptide-based templates^{33–38}, the goal of these bio-inspired approaches was to efficiently control particle shape and size during particle nucleation events, or more commonly, during the growth stage following seed preparation.³⁹ Some studies reveal, for example, that variation in physical size and fluorescence characteristics of CdS nanoparticles can be achieved by adding specific homopolymer sequences during nanoparticle synthesis.⁴⁰ DNA has also been used as a template to create Ag nanoring structures which could not be achieved using conventional direct growth methods.^{41, 42}

Select studies by Wang *et al.* have specifically investigated the effect of incubating soluble oligonucleotides (i.e. not intentionally conjugated to material surface) with gold seeds during the growth stage. Their studies involved incubating solutions of 30 base-long (polyadenine, polythymine, or polycytosine)^{43, 44} or 20 base-long (polyguanine)⁴⁴ homopolymers with CTAB-stabilized gold nanoprism seeds. They reported a variation occurs in particle morphology and topography ranging from rough (polyadenine) and smooth (polycytosine) surfaces to star-shaped (polythymine) and hexagonal (polyguanine)

nanoparticles.⁴³ Depending on sequence compositions, binary mixtures of the homopolymers resulted in intermediate morphologies and surface roughness in the resulting nanoparticles.

While few studies^{43, 44} have investigated particle growth in the presence of nucleic acids, base-specific and structure-specific interactions of oligonucleotides with gold surfaces have been reported. SPR and FTIR analysis on gold surfaces can provide information on relative differences in the timing and extent of oligonucleotide adsorption events.^{45–47} Notably, while bare planar gold serves as a convenient sample geometry for characterizing surface binding events, the nature of these binding events on colloidal gold may be significantly affected by additional materials parameters ranging from curvature to additional surface moieties (e.g. citrate).⁴⁶ For gold nanoparticles initially stabilized by citrate ions, bridging of nanoparticle surfaces via single-stranded homopolymers of DNA has been assessed using colorimetric analysis and UV-Vis spectroscopy.^{48, 49} Collectively, these studies indicate that homopolymer sequences do not have equivalent affinities for gold substrates with purines adsorbing more strongly than pyrimidines to gold in the following order: G>A>C>T.^{45, 48} Among these studies, the secondary structure of DNA (single-stranded chains vs. double-stranded helix) has been shown to affect the propensity for gold nanoparticles to aggregate.^{47, 49}

In the current spectroscopy study, base-specific and structure-specific effects of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) added during seeded growth are investigated to reveal if the presence of various nucleic acids directs the shape evolution of resulting gold nanoparticles. In an effort to directly compare results of adding various nucleic acids ranging from individual nucleotides to mixtures of polynucleotides in the current study, a common gold seed approach involving CTAB was chosen based on the work of El Sayed and coworkers.^{15, 16} While the seed preparation was identical in all cases, one of two growth conditions are used, namely either a gold nanosphere (AuNS) growth solution (aging of the original seed solution) or a gold nanorod (AuNR) growth solution (seed solution added to a separate AuNR growth solution with additives such as silver nitrate). The effects of various nucleic acids on gold nanoparticle formation under these two broad classes of either nanosphere or nanorod growth solutions is primarily investigated using UV-Vis spectroscopy. Due to its widespread and practical use as a characterization tool for gold nanoparticle suspensions, UV-Vis spectroscopy was chosen as our primary analytical tool in order to quantitatively assess differences in the spectral signatures of the resulting nanoparticle suspensions from numerous nucleic acid incubation conditions. In order to identify base specific effects, 20 base-long homopolymer (**A20**, **T20**, **C20**, **G20**) and random (**R20**) sequences alone and as mixtures are employed in these studies. Structure-specific effects (ssDNA vs. dsDNA) were also investigated by incubating gold seeds with mixtures of complementary homopolymer sequences as well as with specific sequences (**S20**, **S20'**) that were shown to be capable of duplex formation (**S20:S20'**) under the nanoparticle growth conditions explored. Pure and mixed monomeric forms of nucleic acids were also investigated as additives during nanoparticle growth.

EXPERIMENTAL SECTION

Chemicals

Hydrogen tetrachloroaurate (III) hydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate (AgNO_3), L-ascorbic acid, hexadecyltrimethylammonium bromide (CTAB), hydrochloric acid (HCl), and deoxynucleoside monophosphates (dNMPs: dAMP, dTMP, dGMP, and dCMP) were purchased from Sigma Aldrich, St. Louis, MO. Sodium borohydride (NaBH_4) was purchased from Fluka Analytical, Bushs, Switzerland. Tris HCl (pH 7.5) was purchased from Amresco, Solon, OH. Deoxynucleoside triphosphates (dNTPs: dATP, dTTP, dGTP, dCTP, dNTP Mix) were purchased from Invitrogen, Grand Island, NY. All DNA sequences were synthesized by Integrated DNA Technologies, Inc., Coralville, IA. Buffers were prepared using 0.2 μM filtered water from a Barnstead Nanopure ultrapure water purification system. 1 mM stock solutions of DNA were prepared using 1 mM Tris HCl, pH 7.5.

Preparation of gold nanoparticle seed solution

Gold nanoparticle seeds were synthesized according to the methods described by Nikoobakht and El Sayed.¹⁶ CTAB solution (20 mL, 0.2 M) was mixed with a solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (20 mL, 0.5 mM). After 30 min of stirring, a freshly prepared solution of ice-cold NaBH_4 (2.4 mL, 0.01 M) was then added to the stirred seed solution and mixed for an additional 2 min. Gold seed suspensions were then added to nucleic acid solutions as described in the next section.

Preparation of gold nanospheres and nanorods

Gold nanospheres were allowed to form (in the absence or presence of various nucleic acids) by allowing the gold seed solution to age for up to seven days. Gold nanoparticle seeds for gold nanorod synthesis were prepared at the same reagent ratios as described above, however, the total reaction volume (including additional NaBH_4) was scaled to 10.6 mL. Using magnetic stir bars, the seed solution was then stirred on a low setting for an additional 2 min before being poured into a separate glass container and set aside for 45 min to 1 hr prior to incubation with (or without) various nucleic acids in a gold nanorod growth solution described next.

A 10 mL gold nanorod growth solution was prepared to provide the same growth conditions for each set of nucleic acids for a given nanoparticle suspension (1 mL each). For cases involving a large number of samples (e.g. kinetics studies over a 7 day timeframe), the total reaction volume was scaled accordingly. Then, for each 10 mL volume, 160 μL of 5 mM AgNO_3 was added to 5 mL of 0.2 M CTAB and stirred on a low setting for 30 s. A 5 mL volume of 1 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was added to the solution and stirred on a low setting for 5 min. A 54 μL volume of 0.1 M L-ascorbic acid was then added to the solution and stirred on a low setting for 5–10 min. To then initiate AuNR growth, 12 μL of a gold seed solution (aged 45 min to 1 h) was added to 10 mL of AuNR growth solution. The solution was immediately stirred on a high setting for 15–20 s and then added to various nucleic acid solutions described below.

Nucleic acid incubation and UV-Vis spectroscopy and TEM analysis

A list of oligonucleotide sequences is provided in Table 1. For all seed suspensions incubated with nucleic acids, each oligonucleotide or nucleotide was incubated at a final concentration of 2 μM in a 1 mL total volume of either (i) the seed solution (to be aged) or (ii) the seed solution added to the AuNR growth solution. For cases involving mixtures of oligonucleotides or nucleotides, each oligonucleotide or nucleotide was added to yield a final concentration of 2 μM for each individual species. Select experiments were also conducted at a nucleic acid concentration of 0.1 μM . Following the addition of seed solutions to a particular nucleic acid solution, suspensions were mixed on a rotomixer and examined at various timepoints using UV-Vis analysis. For the 2 h and 7 d timepoints, UV-Vis spectra were gathered at 1 nm intervals using a Biotek Synergy H1 Hybrid Reader. An exception to this protocol was used for the AuNR kinetics study involving 15-min intervals for the first hour of incubation with nucleic acids. For the early time points in these particular kinetics studies, UV-Vis spectra was collected with a sweeping scan at a 1 nm wavelength resolution. For all other time points occurring at or after 1 h, however, the previously described scanning conditions (i.e. 1 nm intervals) was used. After data collection, spectra for each set of samples were first normalized against a water blank. Next, each sample was normalized to the same relative absorbance value of 0.3 at 400 nm as others have done in previous studies.²⁴ Samples were additionally characterized via transmission electron microscopy. TEM images were acquired with a JEOL 100CX II transmission electron microscope at 100 kV. Samples for TEM were centrifuged (14,000 rpm $\times$ 15 min) twice to remove surfactant and redispersed in nanopure water. After redispersion, 0.3 μL of the sample was drop-cast onto a carbon-coated copper TEM grid and allowed to dry at room temperature.

RESULTS AND DISCUSSION

Effects of 20 base-long homopolymer additions to ageing gold seed suspensions

The effects of incubating 20 base-long homopolymer sequences (**A20**, **T20**, **C20**, or **G20**) as well as random oligonucleotide sequences (**R20**) with a gold nanoparticle seed solution are investigated first. Following a 2 h incubation period, no spectral differences are observed between any ageing nanoparticle suspensions in the presence or absence of single-stranded DNA (ssDNA) as shown in FIG. 1(a). Notably, the featureless spectra at this early time point are likely due to the relatively small seed size (~ 4 nm).¹⁶ After a 7 d incubation period shown in FIG. 1(b), a peak (~ 525 nm) appears for both controls and all of the DNA cases except for polyadenine (**A20**) which induces a red-shift (571 nm) and overall broadening of the characteristic peak. Unlike the other suspensions, visible nanoparticle sedimentation does occur in the presence of **A20** within 7 days. Together, the spectral differences and rapid sedimentation behavior indicate that polyadenine induces particle aggregation not observed with the other homopolymers or random oligonucleotide sequences. This aggregation behavior most likely stems from polyadenine bridging spherical nanoparticles together into disordered clusters as indicated in separate TEM micrographs in Supporting Information (FIG. S1 (a)). Prior work by Wolf *et al.* on planar substrates suggests that 25 base-long polyadenine exhibits the strongest and fastest binding activity to gold surfaces compared to polycytosine and polythymine.⁴⁵ While CTAB surfactant is a necessary additive in the

current study to minimize aggregation of either the seeds or nanoparticles, separate washing studies (results not shown) as well as prior work⁵⁰ indicate that the CTAB is only weakly associated with the nanoparticle surface. Thus, the polyadenine may effectively displace enough of the CTAB bilayer to allow polymer-induced bridging between nanoparticle surfaces. Interestingly, polymer-induced bridging typically occurs under dilute polymer conditions with relatively high molecular weight polymers; however, it is known that relatively short DNA strands (20 bases or less) can participate in forming duplex bridges between nanoparticles.^{30, 51} Thus, nanoparticle bridging by the single-stranded oligonucleotides appears plausible. Nucleobase-dependent agglomeration of gold nanoparticles has also been observed by Storhoff *et al.*, who reported that deoxynucleosides (dA, dG, dC) induced significant shifts in the surface plasmon band frequency due to agglomeration while dT only induced slight changes.⁴⁸ To further investigate the effects of polyadenine on gold nanoparticle ageing effects in the current work, two additional incubation conditions are undertaken. First, while a 2 μ M concentration of oligonucleotides is typically used in the current studies, a 0.1 μ M concentration is also explored in select oligonucleotide cases such as **A20**. Even at this lower polyadenine concentration, however, a red shift and peak broadening effect are observed though to a lesser extent as shown in Supporting Information (FIG. S2(a,b)). Second, while incubation studies typically involved adding freshly-prepared (within 30 min) gold seeds to the various oligonucleotide solutions, supplemental studies demonstrate that the same peak trends occur even if **A20** is not added until the gold nanoparticle seed solution has already aged for 7 d as shown in Supporting Information (see FIG. S3). To further examine the effects of individual bases alone, analogous 7 d incubation studies with the monomeric derivatives of oligonucleotides (e.g. dNTPs, dNMPs) are also carried out as shown in the Supporting Information (see FIG. S4(a,c)). The lack of spectral differences between any of the nanoparticle suspensions in these cases, however, indicates that the polymeric nature of polyadenine plays a key role in affecting spectral behavior and further supports the likelihood of polyadenine-induced nanoparticle bridging.

Effects of 20 base-long homopolymer additions to gold seeds in gold nanorod growth solution

In the next series of DNA incubation studies, freshly-prepared (within 45 min – 1 h) gold nanoparticle seeds are first added to a gold nanorod (AuNR) growth solution and this mixture is then added to various nucleic acid solutions. Following a 2 h incubation time, two peaks are observed to form in either the absence (controls) or presence of DNA as shown in FIG. 1(c). Prior studies indicate that the dual peaks are associated with the transverse and longitudinal axes of AuNR.^{2, 15} Differences between the transverse peak wavelength values (~520 nm) are typically small; however, differences in the longitudinal peak wavelength values (~600–1600 nm) between various AuNR suspensions can be significant and are generally attributed to differences in the aspect ratio of the AuNR (e.g. a lower longitudinal peak wavelength value corresponds to nanorods with a lower aspect ratio⁵²). As compared to the control cases, the most significant spectral differences at the 2 h timepoint involve a modest blue shift in the presence of polythymine, **T20**, and the 20 random base DNA, **R20**, cases. At a lower DNA concentration of 0.1 μ M, however, spectral differences are not as apparent for any of these sequences as shown in Supporting Information (see FIG. S2(c)).

As these suspensions continue to age in AuNR growth solution for 7 d, the longitudinal peak undergoes a blue shift in every case, but to a different extent depending on the sequence as shown in FIG. 1(d). In previous reports this shift to lower longitudinal peak wavelength values with prolonged growth time in the absence of DNA has been attributed to nanoparticle overgrowth resulting in nanorod-like shapes with smaller aspect ratios.⁵³ In particular, the random **R20** sequences appear to ultimately have the most dramatic effect on this blue-shift effect of the longitudinal peak. In fact, while transverse and longitudinal peaks are still evident by the seventh day in the controls and homopolymer cases, these peaks nearly merge in the **R20** case, even at a lower concentration of 0.1 μM (see FIG. S2(d) in Supporting Information). Here, the **A20** strands do not impose the destabilizing effect evident in the aged gold nanoparticle suspensions alone (see FIG. 1(b)). In fact, of all the homopolymers, it is polythymine, **T20**, that has the most dramatic effect on peak shifts over time as shown in FIG. 1(d). Similar to the aged gold nanoparticles studies, analogous incubations for seeds in AuNR growth solution with dNTPs do not result in any significant differences in the dual peaks observed in the UV-Vis spectra as shown in Supporting Information (see FIG. S4(b)). Thus, this result further indicates that the polymeric nature of the oligonucleotides plays a key role in the spectral evolution of these nanoparticle suspensions.

Effects of homopolymer mixture additions to gold seed suspensions

In the next series of studies, specific mixtures of homopolymers are added and suspensions are examined following a 7 d incubation time. In order to evaluate if the observed **A20**-induced aggregation effect on aged gold nanoparticle seeds is mitigated by the presence of other bases, **A20** is first mixed with one other homopolymer (**T20**, **C20**, or **G20**) and then added to seed solutions for a 7 d ageing timeframe. FIG. 2(a) shows the UV-Vis spectra of the resulting suspensions in which the peak broadening effect is increasingly mitigated by the copresence of one other homopolymer in the following order: **C20** < **G20** < **T20** as well as by the copresence of all four homopolymers. In competitive adsorption studies conducted by Kimura-Suda *et al.*, the relative adsorption affinities of 5-mer ssDNA oligonucleotides on gold surfaces was reported to be T (weakest) < G < C < A (strongest).⁴⁷ This trend is comparable to our results in which one can infer that the homopolymer (**A20**) causing nanoparticle aggregation (see FIG. 1(b)) is likely to be the strongest adsorbate. Furthermore, when incubated in the presence of two homopolymers with increasingly weaker binding affinity for the gold substrate (**C20** (strongest adsorbate) > **G20** > **T20** (weakest adsorbate)), the degree of **A20**-induced aggregation also diminishes in the same order in FIG. 2(a). In the case of these homopolymer mixtures, however, one must consider both the DNA-nanoparticle interactions as well as the DNA-DNA interactions. The **A20-T20** mixture is a particular case in which polythymine has the weakest apparent affinity for the nanoparticles, but the strongest affinity for polyadenine. Compared to all other homopolymer combinations, the dual mixture of **A20-T20** promotes the smallest differences in the peak location (530 nm) and breadth compared to the controls. Given the complementarity between strands and the distinctive spectroscopic effect compared to the other homopolymer additions, it is likely that a mixture of **A20** and **T20** strands leads to the formation of partially hybridized duplexes that inhibit single-stranded overhangs or unhybridized segments of a polyadenine “tail” from forming bridges between nanoparticles and causing

the aggregation evident in the pure **A20** case shown in FIG. 1(b). Notably, spectra at an early time (2 h) remain featureless indicating that mixtures of homopolymers, even **A20-T20**, do not promote any significant, immediate differences in the spectra (see FIG. S5 in Supporting Information).

Effects of homopolymer mixture additions to gold seeds in gold nanorod growth solution

Given the faster evolution of peak formation and the shifts in the longitudinal peak wavelength values over time for AuNR growth solution conditions shown in FIG. 1(c,d) compared to that of AuNPs in FIG. 1(a,b), analogous experiments with homopolymer mixtures in AuNR growth conditions were conducted to evaluate the effects of the **T20** sequence on resultant spectra after a 2 h incubation (instead of 7 d). FIG. 2(b) shows that the blue-shift resulting from the presence of **T20** is increasingly suppressed by the copresence of one other homopolymer in the following order: **G20** < **C20** < **A20** as well as by the presence of all four homopolymers. Similar to the aged gold seed solution shown in FIG. 2(a), it is likely that specific base effects of single-stranded polythymine on seed growth in AuNR growth solution is inhibited by Watson-Crick base pair matching in the **T20-A20** mixtures.

Effects of 20 base-long complementary strands alone and mixed together on ageing gold seeds

The next series of DNA incubation studies involves the addition of two complementary sequences (**S20** and **S20'**) consisting of 50% thymine and 50% adenine bases, respectively, as shown in Table 1 and capable of forming **S20:S20'** duplexes under the incubation conditions explored for gold seed formation and nanoparticle growth explored here (see FIG. S6 in the Supporting Information). Similar to the ssDNA incubation studies discussed in the previous section, here the effects of double-stranded DNA (dsDNA) additions on gold nanoparticle formation are explored using UV-Vis spectroscopy as shown in FIG. 3. As before, gold seeds here are aged with various DNA solutions. FIG. 3(a) shows that by the 2 h timepoint, the spectra remain featureless for the ssDNA cases of **S20** or **S20'** alone; however, a peak at 531 nm does appear if both complementary strands are present. The emergence of this peak at this early timepoint indicates that rapid nanoparticle growth occurs in the presence of the duplexes. Moreover, it is clear that this result is due to the presence of **S20:S20'** duplexes and not the single-stranded sequences since no characteristic peaks appear at the 2 h timepoint if gold seeds are incubated with the individual single-stranded **S20** or **S20'** oligonucleotide strands. The early emergence of this peak is also observed at the lower concentration (0.1 μ M) of **S20:S20'** duplexes as shown in Supporting Information (FIG. S7(a)). Notably, this characteristic peak was not evident at early timepoints for the complementary **A20-T20** mixtures (see FIG. S5 in Supporting Information). The differences between these two mixtures may be attributed to differences in the duplex structure since the primary structure or specific sequence of bases in **S20** and **S20'** strands promotes the formation of duplexes with blunt ends while a rich range of partially hybridized duplexes with single-stranded overhangs of varying baselength are possible for an **A20-T20** mixture. As shown in FIG. 3(b) following a 7 day incubation, similar spectra occur in all cases with and without DNA present, with one exception. Similar to the **A20** case shown previously in FIG. 1(b), the adenine-rich **S20'** also causes a peak

broadening effect as evidenced by a red-shift of the characteristic peak from 524 nm (for the controls) to 580 nm (see FIG. 3(b)). Separate TEM micrographs confirm the formation of gold nanospheres in the presence of the **S20:S20'** duplexes (see FIG. S1(b,c) in the Supporting Information).

Effects of 20 base-long complementary strands alone and mixed together on gold seeds in gold nanorod growth solution

In contrast to the early appearance of a single peak in aged gold seed solutions incubated with **S20:S20'** duplexes as described above, gold seed in AuNR growth solution incubated with the **S20:S20'** duplex solutions exhibits a modest blue shift of the longitudinal peak compared to the controls at the 2 h time point as shown in FIG. 3(c). At the 7 d timepoint, however, there was little difference in the translational and longitudinal peak wavelengths (~520 nm and ~620 nm) between the **S20:S20'** case and the control samples with no DNA additions as shown in FIG. 3(d). The thymine-rich **S20** case, on the other hand, promotes the most significant effect on the spectra causing a large blue-shift of the longitudinal peak at the 2 h time point which continues to shift until it ultimately merges with the translational peak to form a single peak at 545 nm at the 7 d timepoint. Intriguingly, this blue-shift effect for this thymine-rich (50%) **S20** sequence is more dramatic than that observed for the pure polythymine case shown in FIG. 3(c,d). Moreover, the adenine-rich (50%) **S20'** sequence causes only a modest blue-shift during the early incubation time in FIG. 3(c), but causes extensive aggregation over the long incubation time shown in FIG. 3(d). The featureless spectra shown in FIG. 3(d) for the **S20'** case is due to the fact that the aggregate ultimately adheres to the reaction vessel wall during the 7 d incubation time and could not be redispersed for spectroscopic evaluation. In contrast to the **S20** and **S20'** cases, the longitudinal peak undergoes a more modest blue-shift in the other cases shown in FIG. 3(c,d) in which two peaks remain evident at all timepoints. The presence of the single peak by day 7 in the **S20** case suggests that the particles are likely to be more spherical in shape as indicated by TEM micrographs shown in Supporting Information (see FIG. S8(a)). As a separate set of experiments, additional incubation studies conducted in the absence of silver nitrate indicate only a single peak occurs at the 2 h and 7 d timepoints in either the presence (see FIG. 3(c,d)) or absence (results not shown) of **S20** strands. Silver ions reportedly promote the anisotropic growth of the CTAB-stabilized gold seeds into gold nanorods through its preferential deposition onto {110} faces during particle growth, effectively inhibiting growth on this plane and promoting growth in the [100] direction on {111} facets.²³ While the formation of nanorods themselves does require the presence of silver nitrate, the presence of DNA sequences such as the thymine-rich **S20** does appear to significantly affect the spectral evolution of gold nanorods and may even inhibit the role of silver nitrate in promoting stable gold nanorod growth over time. In addition, unlike the accelerated growth effects of **S20:S20'** mixtures on gold nanoparticle seed in the absence of AuNR growth solution at both 2 μ M (see FIG. 3(a)) and 0.1 μ M (see FIG. S7(a) in the Supporting Information), a lower concentration of **S20** (0.1 μ M) does not promote significant differences in the AuNR spectra compared to the controls as shown in Supporting Information (FIG. S7(c,d)).

Effects of select ssDNA sequences on spectral evolution of gold nanorod growth solutions

The ssDNA cases (**T20**, **R20**, and **S20**) promoting the most dramatic blue shift in the longitudinal peak wavelengths from studies described above were selected for a more detailed study to monitor peak evolution over several timepoints during the first 2 h (FIG. 4) and first 7 d (FIG. 5). As shown in FIG. 4 (a–d), the characteristic transverse and longitudinal peaks appear within 15 min of adding seeds to AuNR growth solution in either the absence (controls) or presence of DNA. As reported previously by Zweifel *et al.*⁵³ a blue shift of the longitudinal peak of AuNR during growth does occur within the first 2 h in the absence of DNA; however, for the control case shown in FIG. 4(d), only a small shift ($\lambda = 9$ nm) in the longitudinal peak location occurs over this 2 h period. Larger shifts, on the other hand, are observed in the presence of DNA for the **S20** ($\lambda = 32$ nm), **T20** ($\lambda = 28$ nm) and **R20** ($\lambda = 17$ nm) cases. These suspensions involving **S20**, **T20**, or **R20** additions were then monitored daily over a 7 d period as shown in FIG. 5(a–d). Within 1 d, **S20** induces the most dramatic blue shift in its longitudinal peak wavelength ($\lambda = 114$ nm) as compared to its 15 min time point value. The dramatic blue shift continues for the **S20** case until this longitudinal peak merges with the translational peak to form a single peak (535 nm) within 3 days. A similar merger of peaks (533 nm) occurs by day 5 for the **R20** case. While a blue shift of the longitudinal peaks continues for the **T20** and control cases, two distinct peaks still remain apparent by day 7 for these two cases. For more facile comparison, the relative change in the longitudinal peak wavelength value over time is plotted in FIG. 6 using spectra presented in FIG. 4 and FIG. 5 for the **S20**, **R20**, **T20**, and control cases. As shown in FIG. 6, larger blue-shifts in the longitudinal peak wavelength values are generally observed in the presence of any of these DNA sequences than in the absence of DNA, particularly at later time points. Of these DNA sequences, the shift in peak values occurs most rapidly for the **S20** case. In contrast to the shifts observed for the longitudinal peak, the transverse peak (ranging from 505 to 530 nm, depending on suspension conditions) remains relatively unchanged over time for a given suspension. This result is not surprising since the transverse peak is reportedly relatively insensitive to differences in the aspect ratio of gold nanorods.⁵⁴ Additionally, it has been shown that suspensions with dispersed gold nanospheres with a size range of 5–20 nm have a similar characteristic peak value (~520 nm).⁵⁵

In addition to the spectral analysis in which nanoparticle shape information can be inferred, TEM was performed following a 3 d incubation with these select sequences as shown in Supporting Information (FIG. S8). As expected for the **S20** incubation case in which only a single UV-Vis peak was observed for the nanoparticles, only spherically-shaped nanoparticles are found (see FIG. S8(a,e) in the Supporting Information). The **T20** incubation case yields mostly nanorods with a few larger spherical and oblong particles (see FIG. S8(b) in the Supporting Information) while **R20** and the control (no DNA) resulted primarily in oblong nanoparticles with a small aspect ratio (see FIG. S8(c) and (d) in the Supporting Information).

CONCLUSIONS

In the current work, monomeric forms of the nucleic acids do not appear to affect the spectral evolution of growing nanoparticles. In contrast, base-specific and structure-specific effects of 20 base-long oligonucleotides are evident in several cases involving seed-mediated growth of gold nanoparticles. Moreover, while the distinctive and likely dynamic role of the cationic CTAB species is not clear, separate washing studies indicate that CTAB is only weakly bound to the gold surface. Thus, displacement by a stronger adsorbate such as polyadenine to cause aggregation in the aged gold nanoparticle seed is possible. Among the homopolymers, modest base-specific effects are observed in terms of the timing and extent of the blue-shift of the longitudinal peak of the gold nanorods in the case of polythymine. For either nanoparticle case, however, the copresence of the complementary homopolymer lessens the effect of the homopolymer alone. In fact, the secondary structure of the DNA appears to play a key role in the evolution of the nanoparticle spectra since the presence of blunt-end duplexes has a marked effect on accelerating nanoparticle growth in an aging gold seed solution. This accelerated growth, however, was not observed for mixtures of complementary homopolymers which can likely form a heterogeneous population of short hybridized segments (of varying baselength) flanked by single-stranded segments. Collectively, the interactions between complementary oligonucleotides involving Watson-Crick base pair matching appears to either completely overcome (for blunt-ended duplexes) or at least reduce (for duplexes with single-stranded overhangs) interactions between oligonucleotides and nano-sized gold particles during their growth. Ongoing work involves competitive adsorption studies between nucleic acids on various gold surfaces to further elucidate any relative affinity differences arising from base-specific as well as structure-specific effects on gold-DNA interactions.

Supplementary Material

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FIG. 1.

UV-Vis spectra of (a,b) gold seed and (c,d) gold seed in AuNR growth solution following incubation with various 20 base-long homopolymers (A20, T20, C20, G20) and random (R20) sequences for 2 h (left) and 7 d (right). Controls involve the addition of 2 μ L of Tris HCl (Ctr1) or 2 μ L of nanopure water (Ctr2) in the absence of DNA. The resulting peak wavelength values are included in the legend.

FIG. 2.

UV-Vis spectra of (a) gold seeds after a 7 d incubation and (b) gold seed in AuNR growth solution after a 2 h incubation in the presence of various homopolymer mixtures of two or four sequences. Controls involve the addition of Tris HCl (Ctr1= 4 μ L Tris HCl; Ctr2= 8 μ L Tris HCl) or water (Ctr3= 4 μ L nanopure water) in the absence of DNA. The resulting peak wavelength values are included in the legend.

FIG. 3.

UV-Vis spectra of (a,b) gold seeds and (c,d) gold seeds in gold nanorod growth solution following incubation with various complementary ssDNA alone (**S20** or **S20'**) and mixed together (**S20:S20'**) for 2 h (left) and 7 d (right). Controls involve the addition of Tris HCl (Ctr1= 4 μ L Tris HCl; Ctr2= 2 μ L Tris HCl) or water (Ctr3= 2 μ L nanopure water) in the absence of DNA. The resulting peak wavelength values are included in the legend. The letters "NA" in this legend for **S20'** in (d) indicates that the suspension spectra could not be measured due to formation of an aggregated pellet that could not be redispersed on day 7.

FIG. 4.

UV-Vis spectroscopy over the first 2 h (in 15 min intervals) of gold seeds in AuNR growth solution incubated with the following solution additions: (a) **S20** (b) **T20** (c) **R20** or (d) 2 μ L Tris HCl only (no DNA). The resulting peak wavelength values are included in the legend.

FIG. 5.

UV-Vis spectroscopy over the first 7 d (1-day intervals) of gold seeds in AuNR growth solution incubated with the following solution additions: (a) **S20** (b) **T20** (c) **R20** or (d) 2 μ L Tris HCl only (no DNA). The resulting peak wavelength values are included in the legend.

FIG. 6. Relative difference between gold nanorod longitudinal peak values over time relative to the first observed peak time point (at 15 min) for various DNA sequence incubation conditions.

Table 1

List of 20 base-long DNA sequences incubated with gold seeds in the presence or absence of AuNR growth solution.

Nomenclature	Sequence (5' → 3')
A20	AAA AAA AAA AAA AAA AAA AA
T20	TTT TTT TTT TTT TTT TTT TT
C20	CCC CCC CCC CCC CCC CCC CC
G20	GGG GGG GGG GGG GGG GGG GG
R20	25% (A,T,C,G) in random order
S20	TTT TTT GGA TTG CGG CTG AT
S20'	ATC AGC CGC AAT CCA AAA AA