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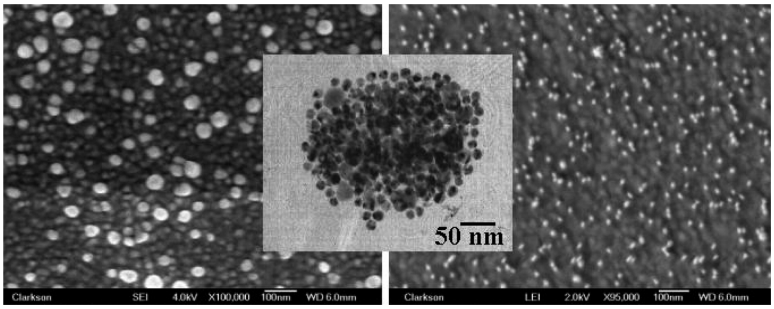

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Hybridization of Oligonucleotide-Modified Silver and Gold Nanoparticles in Aqueous Dispersions and on Gold Films

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Abstract: Functionalization of silver and gold nanoparticles by 12mer-thiolated homo-oligonucleotides, SA and ST (containing only adenine or thymine, respectively), and their hybridization and dehybridization in aqueous dispersions have been described. In addition, ST and SA were self-assembled onto gold films and hybridized with their complementary pairs, unlabeled or labeled by gold and silver nanoparticles. The base pairing between DNA strands and the types of oligonucleotides (adenine or thymine) attached to the nanoparticles was detected by Polarization Modulated Fourier Transform Infrared Reflection Absorption Spectroscopy (PM-FTIRRAS).

Introduction

Oligonucleotide-derivatized nanoparticles have been extensively employed for detecting DNA hybridization in aqueous dispersions and on surfaces.[1−7] The overwhelming majority of the use of gold nanoparticles is based on their stability, relative ease of preparation, functionalization, and detection (by absorption or scattering spectroscopy of the intense localized surface plasmon resonance, LSPR). The extreme sensitivity of the bandwidth, the peak height, and the position of the absorption (or scattering) maximum of LSPR spectra to environmental changes has prompted the development of gold nanoparticle-based sensors,[8−11] including those which directly monitor DNA hybridization.[5,7,12−14] For multiple assays, however, it would be highly desirable to have a second marker of different color.[15] The simplest choice is the exploitation of silver nanoparticles whose LSPR absorption (or scattering) maximum occurs at a wavelength different from that of the gold nanoparticles. Unfortunately, functionalization of silver nanoparticles by oligonucleotides has proved to be particularly difficult[16] and, to the best of our knowledge, so far has eluded the experimentalists.

We report here the successful modification of silver and gold nanoparticles by short oligonucleotides of homogeneous sequence, containing only adenine or thymine. Specifically, we employed 5′-disulfide-functionalized 12mer oligo(d)T (ST) and 5′-disulfide-functionalized 12mer oligo(d)A (SA) to functionalize silver and gold nanoparticles (herein referred to as Ag/ST, Au/ST, Ag/SA, and Au/SA) and to hybridize the oligonucleotide-modified silver and gold nanoparticles (to Ag/ST−SA/Ag, Au/ST−SA/Au, and Au/ST−SA/Ag aggregates). We describe the absorption spectroscopic changes which accompany the hybridization and dehybridization of Ag/ST−SA/Ag, Au/ST−SA/Au, and Ag/SA−ST/Au aggregates in aqueous dispersions. In addition, we have self-assembled ST and SA onto gold films and detected their hybridization with Ag/SA, Au/SA, and Au/ST by Polarization Modulated Fourier Transform Infrared Reflection Absorption Spectroscopy (PM-FTIRRAS).

PM-FTIRRAS is a highly sensitive technique, capable of determining the absorption of ultrathin self-assembled monolayers (mercaptopropionic acid, for example[17]). In PM-FTIRRAS, polarized and modulated light is focused on a sample at a grazing incident angle. The reflected light is then demodulated into the sum, and the difference of p- and s-polarized light and their dichroic ratio is used to obtain the spectrum.[17] An additional important advantage of PM-FTIRRAS is that it requires no background (or reference) and water vapor, and CO_2 peaks are practically eliminated from the spectrum.[18,19]

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In addition to the determination of oligonucleotide hybridizations[20] by PM-FTIRRAS, we showed this technique to be capable of distinguishing between the types of the oligonucleotides (only adenine or thymine) undergoing hybridization.

Experimental Section

Materials. The 12mer oligonucleotides (ST and SA, containing only thymine or adenine, respectively) with a 5′-thiol modification ($OPO_3-(CH_2)_6-S-S-(CH_2)_6OH$) were purchased (Integrated DNA Technologies, Inc.) and used as received. Gold chloride trihydrate, sodium citrate, Tris-HCl, EDTA (SIGMA), silver nitrate (Spectrum, Chemical MFG), 1-mercapto-6-hexanol (Alfa Aesar), sulfuric acid, hydrogen peroxide (30%), sodium hydroxide, sodium chloride (Fisher), chrome-plated tungsten rods (CRW, 2 × 0.07″, R. D. Mathis Company), gold shots (6.35 mm and down, semispherical Premion, 99.999%, Alfa Aesar), Fisher *finest premium* microscope slides (BK7, 3′ × 1″, Fisher Scientific), and a nylon bottle-top filter system with the pore size of 0.2 mm (Corning) were used as received. Ultrapure water, with resistivity >18.3 Ω/cm^2 was obtained from a Millipore Milli-Q column system provided with a Milli-pak filter of 0.22 μm pore size at the outlet.

Preparation of Nanoparticles. The gold nanoparticles were prepared by the standard citrate method.[21] Briefly, 200 mL of 0.01% (w/v) $HAuCl_4$ was brought to a boil, and then 7 mL of 1% (w/v) aqueous trisodium citrate was added under vigorous stirring. The color changed into grayish-black and then into wine-red within a few minutes. The dispersion was allowed to cool and filtered through a 0.2 μm pore size nylon bottle-top filter system. The 11.7 ± 1.9 nm diameter gold nanoparticles had a surface plasmon absorbance maximum at 518 nm.

Silver nanoparticles of 24.8 ± 11.6 nm diameter were prepared by reduction of $AgNO_3$ with EDTA.[22] Briefly, 200 mL of 1.6×10^{-4} M EDTA and 0.8 mL of 1 M NaOH was brought to boil, and then 2.0 mL of 2.6×10^{-2} M $AgNO_3$ was added under vigorous stirring. A faint yellow color appeared and intensified after a couple of minutes. The colloid was allowed to cool and filtered through a 0.2 μm nylon bottle-top filter system. The silver nanoparticles had a strong absorbance at 406 nm.

Modification of Au Nanoparticles by Oligonucleotides. The silver and gold nanoparticles functionalized by ST and SA will be called Ag/ST, Au/ST, Ag/SA, and Au/SA, and the nanoparticle complexes formed through the hybridization of surface-attached complementary oligonucleotides will be referred to as Ag/ST−SA/Ag, Au/ST−SA/Au, and Au/ST−SA/Ag aggregates. In a typical derivatization, 1.0 mL of the Au nanoparticle dispersion was incubated with the disulfide-protected oligonucleotide (SA or ST, at 1 μM final cc) overnight. Then, 20 μmol of NaCl was added, and the volume was slowly reduced to 250 μL by slow (1000 rpm) centrifugation of the open Eppendorf tubes at 40 °C, over 10 h, while continuously sucking the air out of the centrifuge chamber. After another overnight incubation, 60 μmol of NaCl was added to 750 μL of water, and the volume was again reduced to 250 μL, as described previously. Unbound oligonucleotides were then removed by repeated centrifugation (13 000 rpm, 30 min) and suspension of the pellet in 1 × TE (10^{-2} M Tris HCl, 10^{-4} M EDTA, pH 7.20), with the desired concentration of NaCl. The gold nanoparticles functionalized by ST and SA will be referred to as Au/ST and Au/SA, respectively.

Modification of Ag Nanoparticles by Oligonucleotides. Derivatization of Ag nanoparticles with ST and SA required a more gentle approach than that used for Au nanoparticles. The NaCl concentration was increased extremely slowly. Typically, 1.0 mL of the nanoparticle dispersion was incubated with the disulfide-protected oligonucleotide (1 μM final cc) for 2 h, then 10 μmol of NaCl was added to 500 μL of water, and the volume was reduced to 0.75 mL by slow (500 rpm) centrifugation of the open Eppendorf tubes at 25 °C, overnight, while continuously sucking the air out of the centrifuge chamber. Then, the volume was adjusted to 1.5 mL by water, and 10 μmol of NaCl was added again, followed by the reduction of the volume to about 0.75 mL by the same way. Then, the volume was adjusted to 1.5 mL by water and 20 μmol of NaCl was added, followed by the reduction of the volume to about 0.75 mL by the same way. After an overnight incubation, the volume was adjusted to 1.5 mL by water; 35 μmol of NaCl was added, and the volume was reduced to 1 mL, as described previously. Unbound oligonucleotides were then removed by centrifugation (15 000 rpm, 30 min, 15 °C) and suspension of the pellet in 1.5 mL of 1 μM disulfide-protected oligonucleotide, again, in the presence of 120 μmol of NaCl. The volume was reduced to about 0.75 mL as described previously, then adjusted to 1.5 mL, and 120 μmol of NaCl was added. The water was slowly evaporated again to reach the volume of 0.75 mL. The sample was stored at room temperature and centrifuged (to remove the unbound oligonucleotides, at 15 000 rpm, 15 °C, for 30 min) before use. The pellet was resuspended in 1 × TE (10^{-2} M Tris HCl, 10^{-4} M EDTA, pH 7.20), with the desired concentration of NaCl. The absorption spectra confirmed the stability of particles. The silver nanoparticles functionalized by ST and SA will be referred to as Ag/ST and Ag/SA, respectively.

Hybridization of Nanoparticles and Melting of the Aggregates. The oligonucleotide-functionalized nanoparticles were hybridized by mixing an equal number of Ag/ST and Ag/SA, Au/ST and Au/SA, or Ag/SA and Au/ST in 1 × TE buffer, containing the indicated NaCl concentration. The nanoparticle complexes formed through the hybridization of surface-attached complementary oligonucleotides will be referred to as Ag/ST−SA/Ag, Au/ST−SA/Au, and Au/ST−SA/Ag aggregates. The melting experiments were carried out in a double-walled glass microflask of 0.6 mL volume by increasing the temperature of water circulating around the sample chamber, 1 °C/step, holding 1 min at each step before measuring the spectrum.

UV−Vis Absorption Spectroscopy. The absorption spectra of colloids were recorded on a single-beam, microprocessor-controlled diode array spectrophotometer with collimating optics (Hewlett-Packard 8452A). The wavelength range and the resolution of the instrument were 190−820 nm (UV−vis) and 2 nm, respectively.

Transmission Electron Microscopy. Transmission electron micrographs (TEM) of the nanoparticles and aggregates were taken by a JEOL JEM-1200EXII electron microscope operating at 120 kV. The supporting grids were Formvar-covered, carbon-coated, 200-mesh copper grids.

Fabrication of Gold Films. The gold film was evaporated on a BK7 glass slide using an Edwards AUTO 306 compact vacuum coater. The pressure during the deposition was 10^{-6} Torr (i.e., 0.76×10^{-9} atm). The thickness and deposition rate of the films were monitored with a built-in system consisting of a quartz crystal mechanically oscillating at its natural resonance frequency (6 MHz). The film thickness was calculated from the change of oscillation frequency of the quartz crystal displayed on a frequency counter and was found to be ∼100 nm. The rate of deposition was 0.5 nm/s. The gold films were always cleaned with freshly prepared piranha solution (75% sulfuric acid, 25% of 30% hydrogen peroxide) immediately before use. *Caution: piranha solution reacts violently with organics!*

Self-Assembly of Thiolated Oligonucleotides on Gold Films. The piranha-cleaned gold films were exposed to 1 μM SA or ST in TE buffer and 100 mM NaCl overnight. After the mixture was thoroughly washed with water, the gold slides were immersed in a 1 mM aqueous solution of mercaptohexanol for 1 h, followed by another step of washing with water and drying with gaseous nitrogen.

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Hybridization of Oligonucleotide-Functionalized Nanoparticles to the DNA Films. The oligonucleotide films were exposed to Au or Ag nanoparticles, functionalized by the complementary oligonucleotide in 1 × TE and 100 mM NaCl, overnight. After hybridization, the gold slide was thoroughly washed with water and dried with gaseous nitrogen.

Polarization Modulated Infrared Reflection Absorption Spectroscopy. All of the spectra were recorded on a Digilab FTS 7000 spectrometer, using a globar tungsten halogen source and a liquid nitrogen-cooled narrow band MCT detector. In all of the experiments, a gold grid polarizer was used to obtain either s- or p-polarized radiation. The aperture (2 cm^{-1}) resolution (8 cm^{-1}) and sensitivity (1) were the same in all of the experiments. The external reflection of the IR beam from the gold film was measured at a grazing angle (~80°). The IR radiation was phase-modulated at frequencies of 400 Hz and at an amplitude of 1 λHeNe while stepping at 1 Hz. The undersampling ratio was 4, and a UDR 4 filter was used. The polarized radiation was modulated by a HINDS ZnSe photoelastic modulator (PEM) operating at 37 kHz and at 0.5 λ (strain axis 45° to the polarizer) before hitting the sample. Each spectrum is a result of one single scan with a spectral range of 400−4000 cm^{-1}. The digital signal processing (DSP) incorporated into the BioRad spectrometer software was used to obtain the spectra. This instrument allowed the gold film supporting the monolayer to also be the reference to obtain the absorption spectra.

Scanning Electron Microscopy. The SEM images were taken by a JEOL JSM-7400F instrument.

Results and Discussion

Functionalization of Silver Nanoparticles by Thiolated Homo-oligonucleotides. The established protocol for the modification of the gold nanoparticles consists of introducing the thiolated oligonucleotides into their colloidal dispersion, typically for 12−16 h, followed by a 100 mM increase of NaCl concentration every 24 h. These conditions appear to destroy the Ag nanoparticles.[1,16] In particular, ion-capped silver nanoparticles are extremely sensitive to an increase of the salt concentration during the immobilization of the thiolated DNA. Generally, charge-stabilized colloidal dispersions coagulate upon addition of salt. Without the cations, however, the DNA strands cannot get close enough to each other to cover the surface sufficiently. Therefore, the successful modification of silver nanoparticles by thiolated DNA requires delicate balancing of the NaCl concentration and the concentration of immobilized oligonucleotides. Recently, the standard method was improved by placing the DNA samples contained in open Eppendorf tubes into a slowly spinning vacuum centrifuge, allowing the slow evaporation of water and, hence, a very gradual increase of the NaCl and oligonucleotide concentrations.[23] By taking advantage of this slight modification and the very gentle and gradual increase of NaCl concentration, we have managed to functionalize EDTA-stabilized 25 nm diameter silver nanoparticles by ST and SA. It has to be noted, however, that the optimization of this method requires considerable trial and error experiments. In our experience, it is very important to reach and maintain at least a 300 mM NaCl concentration to obtain stable Ag/SA and Ag/ST. Prolonged storage (more than several hours) in a low-salt environment results in the loss of immobilized oligonucleotides and particle stability so that they will not hybridize, or if they will, then aggregation is irreversible.

Another important observation is the sequence dependency of successful functionalization. While the SA can be readily attached to Ag nanoparticles, samples with ST require considerably greater care. The silver colloids with ST generally fade (absorption maximum of their surface plasmon spectrum decreases) during the increase of salt concentration, and sometimes the silver nanoparticles fall apart during the washing step. Altogether, the yield of the successfully functionalized Ag/ST is significantly lower than it is with SA, but the surviving particles are stable enough to conduct our experiments. It is well-known from previous studies that the binding affinity of deoxynucleosides to gold nanoparticles depends on the nucleobase present (being highest for adenine and lowest for thymine), but the tendency appeared to be quite the opposite for gold nanoparticles than that for silvers; Au/ST nanoparticles were reported to have an order of magnitude higher stability toward electrolytes.[24] The enhanced stability was attributed to an increase in surface coverage, which enhances surface charge and steric stability.[24]

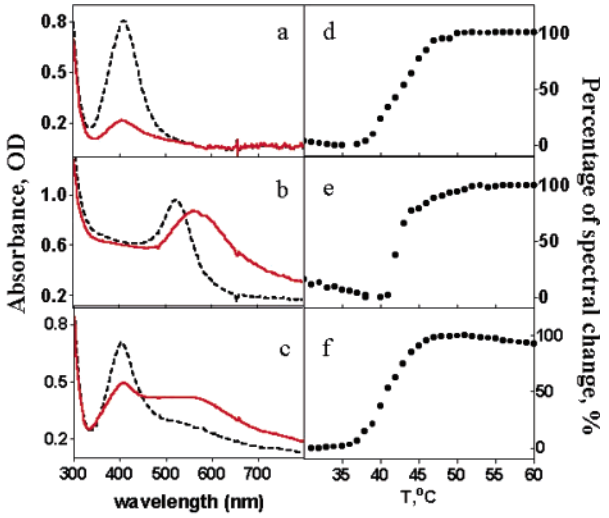

Figure 1. Optical changes upon hybridization and dehybridization of oligonucleotide functionalized Ag and Au nanoparticles. Left side: visible spectrum of Ag/ST−SA/Ag, Au/ST−SA/Au, and Ag/SA−ST/Au aggregates (a, b, and c, respectively) linked by DNA (solid line) and dehybridized by elevated temperature (dashed line). Right side: melting (dehybridization) curves for the same Ag/ST−SA/Ag, Au/ST−SA/Au, and Ag/SA−ST/Au aggregates (d, e, and f, respectively). The change of absorbance was monitored at 406 nm (d and f) and 520 nm (e). All measurements were done in 1 × TE buffer (10^{-2} M Tris HCl, 10^{-4} M EDTA, pH 7.20), 200 mM NaCl.

Optical and Hybridization Properties of DNA-linked Au−Au, Ag−Ag, and Au−Ag Aggregates in Dispersion. Mixing of gold and/or silver nanoparticles, derivatized by complementary oligonucleotides (at the ratio of 1:1 in 1 × TE buffer, containing 200 mM NaCl), resulted in the formation of particle aggregates of different sizes, as confirmed by absorption spectroscopy and TEM (Figures 1 and 2, respectively). The spectral changes upon hybridization of Ag/ST and Ag/SA are strikingly different from those of Au/ST and Au/SA (Figure 1a). The formation of DNA-linked aggregates manifested itself mainly in the substantial decrease of intensity of surface plasmon peak and a small shift of its maximum (from 406 to 410 nm). In the case of the hybridized gold nanoparticles, on the other hand, the shift of surface plasmon peak maximum (from 520 to 564 nm) is clearly dominant over the decrease of peak

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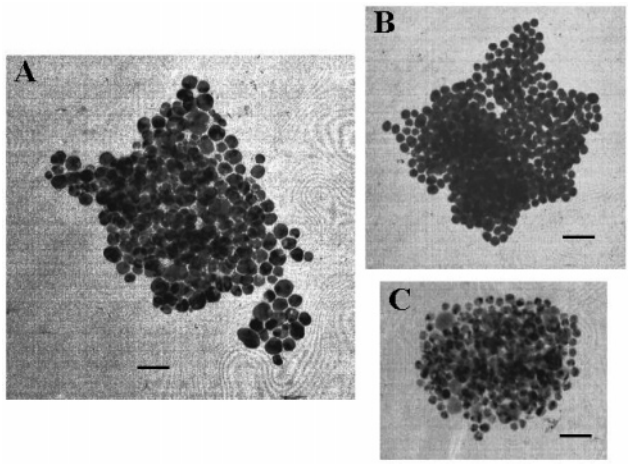

Figure 2. TEM images of silver and gold nanoparticles linked by oligonucleotides. The aggregates are composed of Ag/ST and Ag/SA (A), Au/ST and Au/SA (B), and Ag/SA and Au/ST (C). Scale bar is 50 nm.

intensity (Figure 1b). As could be expected, the spectrum of hybridized Ag/SA and Au/ST combines both types of spectral changes; a significant dampening of the silver SPR peak and a shift of the gold SPR peak mark the process of particle aggregation (Figure 1c). Our results of DNA-linked Au nanoparticles are in full agreement with the previously published studies.[2,5] As we stated earlier, to the best of our knowledge, silver nanoparticles have not been functionalized by thiolated oligonucleotides before. However, the behavior of our oligonucleotide-modified Ag particles is very similar to that published for core−shell Ag/Au nanoparticles (silver nanoparticles enclosed in a thin gold shell and derivatized by oligonucleotides).[16] These core−shell particles had the characteristic silver surface plasmon peak, although lightly dampened, and a weak contribution from gold plasmon at 500 nm. Upon hybridization, their spectra showed a significant decrease in silver-like SPR peak intensity, similar to that in our DNA-linked Ag−Ag and Ag−Au aggregates.[16]

Dehybridization of nanoparticle-attached oligonucleotides (melting) at temperatures higher than 50 °C (see melting curves) resulted in the full restoration of the spectra of separated particles. The melting curves of all three types of DNA-linked aggregates (Ag/ST−SA/Ag, Au/ST−SA/Au, and Ag/SA−ST/Au) exhibited relatively sharp slopes (compared to the melting curves observed in the absence of attached metallic nanoparticles) and high T_m (middle point of spectral changes) characteristic for oligonucleotide conjugated metallic nanoparticles.[3,5,12,13] In comparison, however, the melting curve of hybridized AgST and AgSA had slope more gentle than that for the Au/ST−SA/Au and Au/ST−SA/Ag aggregates. Previous studies have attributed the sharp melting curve to a cooperative mechanism, resulting from the presence of multiple DNA linkers between each pair of nanoparticles.[25] The melting properties of DNA-linked nanoparticles are affected by a number of factors, including DNA surface density, nanoparticle size, interparticle distance, and salt concentration.[25] Considering that the salt concentration and the interparticle distance were the same for all aggregates, and the larger size of silver particles should promote a sharper transition, the broader transition width for

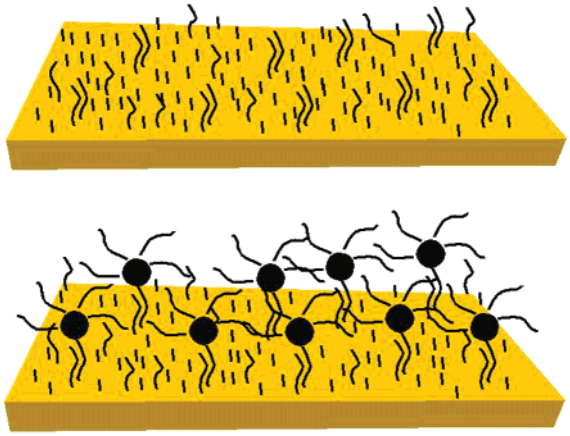

Figure 3. Schematics of the hybridization of oligonucleotides unlabeled (top) or labeled by silver or gold nanoparticles (bottom) to a mixed film of their complementary pair and 1,6-mercaptohexanol, MCH, self-assembled onto a 100 nm thick gold film. The longer, squiggly lines represent the 12mer oligonucleotides, SA or ST, and the straight short lines represent the MCH spacer molecules. Not drawn to scale.

the Ag/ST−SA/Ag assemblies suggests that the particle binding involves less ligands (i.e., the DNA surface density is likely to be much poorer on Ag/ST particles than on Au/ST nanoparticles). The T_m of the three aggregates is comparable: 43 °C for Ag/ST−SA/Ag, 42.5 °C for Au/ST−SA/Au, and 41 °C for Au/ST−SA/Ag.

The TEM images of Ag/ST−SA/Ag, Au/ST−SA/Au, and Au/ST−SA/Ag aggregates also appeared similar to those described for DNA-linked gold nanoparticles, showing mostly smaller and larger three-dimensional structures assembled from distinct nanoparticles (Figure 2).[26]

PM-FTIRRAS of Nanoparticle-Labeled DNA Hybridization. For the detection of hybridization of Ag/ST, Au/ST, Ag/SA, and Au/SA on a gold surface by PM-FTIRRAS, first ST and SA were self-assembled onto a 100 nm thick gold film. These oligonucleotides are linked to a mercaptohexanol (MCH) molecule through the disulfide group (see Experimental Section); therefore, the self-assembly is expected to result in a mixed monolayer composed of DNA and MCH. Nevertheless, to make sure that no bare spots remained on the gold surface, where the oligonucleotides could nonspecifically attach, the film was additionally exposed to a 1 mM solution of MCH for 1 h,[27] prior to allowing it to hybridize by the complementary oligonucleotides, unlabeled or labeled by metallic nanoparticles (Figure 3). Since the sequence of all DNAs consists of thymine or adenine only, it is straightforward to compare the immobilization, hybridization, and spectral features of SA and ST upon interaction with each other and with gold surfaces. The IR absorption peak assignments were based on published data[20,28,29] and are summarized in Table 1.

Figure 4 shows the PM-FTIRRAS spectrum of the film containing the mixture of single-stranded ST oligonucleotide (thymine only) and MCH (spectrum 1). Hybridization of the complementary unmodified 12mer, A (adenine only), with ST manifests itself in the appearance of a broad peak in the region

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Table 1. Assignments of PM-FTIRRAS Peaks of Oligonucleotide Films (based on refs 20, 28, and 29)

assignments	peak position (cm^{-1})
C═O	1702
hybridization	1646
exocyclic NH_2 in adenine	1598−1606
NH_2 of adenine, hybridized	1550−1570
purine and pyrimidine rings	1457−1661
C−O stretching	1378−1380
asPO2-, deoxyribose, C−N	1235−1260
sPO2-	1105−1122
C−O stretch of DNA backbone	1048−1082
P−O stretching	950−955
sugar conformations	891−900, 795−810

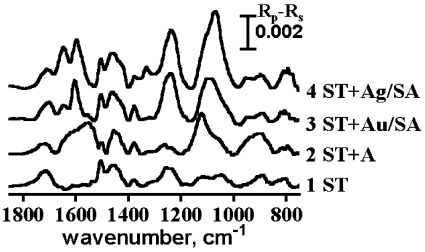

Figure 4. PM-FTIRRAS spectrum of oligonucleotide films. (1) Mixed monolayer of single-stranded 12mer oligonucleotide (ST) and 1,6-mercaptohexanol. (2) ST hybridized with its nonthiolated complementary pair, A. (3 and 4) ST hybridized with Au and Ag nanoparticles, respectively, functionalized by the thiolated complementary pair, SA.

of 1550−1650 cm^{-1} (Figure 4, spectrum 2). This peak is likely to be a combination of the hybridization signature[20] and the exocyclic NH_2 band, characteristic of adenine. All other changes (peak intensity changes between 1300 and 800 cm^{-1}) concern the DNA backbone (see Table 1) and can be explained by the formation of a double strand (i.e., the addition of a second strand and the change of the orientation of the composing molecules). By its very nature, PM-FTIRRAS is very sensitive to the orientation of surface-attached molecules, and for this reason, it can only be regarded as a semiquantitative method.

The spectral changes upon hybridization of Au/SA and Ag/SA to the submonolayer of ST on gold films are clearer and more robust than that for the complementary oligonucleotide unlabeled by metallic nanoparticles (Figure 4, compare spectra 3 and 4 with spectrum 2). Two very distinct new peaks appear at 1646 cm^{-1} and around 1600 cm^{-1}. The peak at 1646 cm^{-1} is assigned to the formation of hydrogen bonds, as it was suggested in a recent study.[20] The second peaks appear at 1606 and 1598 cm^{-1} for Au/SA and Ag/SA, respectively, and are assigned to the exocyclic NH_2 band in adenine. Compared to the peak in the spectrum of hybridization with unlabeled oligonucleotide (Figure 4, spectrum 2), this adenine-specific peak is located at lower frequencies and is much closer to that described for the pure, unhybridized adenine. This discrepancy is explained by the presence of free oligonucleotides that cover the surface of nanoparticles and are not involved in the hybridization (Figure 3). The NH_2 groups of unlabeled oligo(d)A are all constrained due to the base pair formation and their IR absorbance shifts to higher frequencies.[20] The changes in the backbone region also suggest the presence of more unhybridized oligonucleotides attached to the nanoparticles.

Hybridization of SA, self-assembled onto a gold film (with MCH), gave unexpected results (Figure 5). The unlabeled complementary oligonucleotide, T, and Ag/ST were unable to

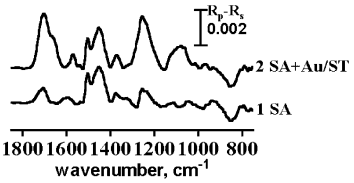

Figure 5. PM-FTIRRAS spectrum of oligonucleotide films. (1) Mixed monolayer of single-stranded 12mer oligonucleotide (SA) and 1,6-mercaptohexanol (MCH). (2) SA hybridized with Au nanoparticles, functionalized by the thiolated complementary pair, ST.

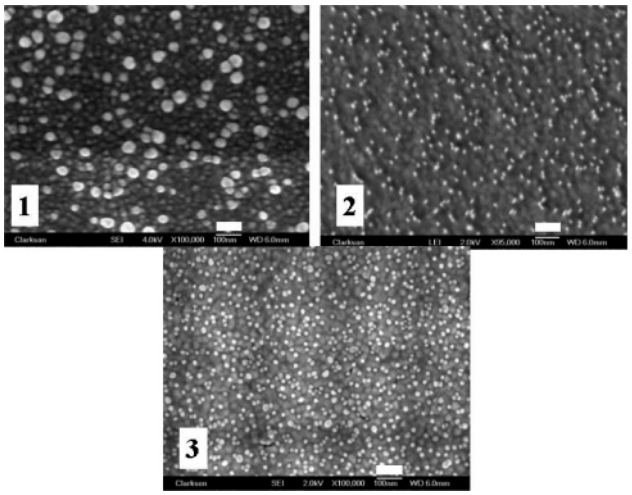

Figure 6. SEM images of oligonucleotide-functionalized Au and Ag nanoparticles, hybridized to their self-assembled complementary pair (SA or ST), self-assembled onto a 100 nm thick gold film. (1) Ag/SA hybridized to ST film. (2) Au/SA hybridized to ST film. (3) Au/ST hybridized to SA film. Scale bar is 100 nm.

make the hydrogen bonds with the single-stranded SA. The spectra remained unchanged, and the SEM showed no particles attached to the gold film (not shown). These results strongly support the recent observation that the high adsorption affinity of oligo(d)A to planar gold surfaces can effectively compete not only with its hybridization to oligo(d)T but also with chemisorption of ST.[30] In our study, the self-assembled SA film was first exposed to the short thiolated spacer molecule, MCH (in order to replace the electrostatic adsorption of the DNA with the strong Au−S bond), then to the complementary oligo(d)T used for hybridization. The negative results indicate that neither the thiol group nor the hydrogen bonds could make the oligo(d)A strands desorb from the surface. On the other hand, the Au/ST hybridized very efficiently with the SA film (Figure 5, spectrum 2). In fact, the coverage by these particles turned out to be twice as high (690 particles/μm^2) as that by Au/SA on ST film (319 particles/μm^2; see SEM images in Figure 6). On the basis of the cooperative binding theory,[24] we hypothesize that the high density of oligonucleotides on the Au/ST particle and the gold film generated multiple hybridization events, enabling a stronger interaction than a single oligonucleotide or a poorly loaded Ag/ST particle. In addition, the Au/ST sample could provide the attractive forces of the gold particles as well as the hydrogen bonding with the complementary oligonucleotides. These forces together are likely to induce the desorption of oligo(d)A from the gold film.

(30) Kimura-Suda, H.; Petrovykh, D. Y.; Tarlov, M. J.; Whitman, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 9014−9015.

Analysis of the spectrum of Au/ST hybridized to the film of its complementary pair reveals the clear signature of thymine at 1702 cm^{-1} (assigned to the carbonyl group). The formation of double strands (i.e., hybridization) is indicated by the peak at 1658 cm^{-1}. Also, we note the shift of the NH_2 absorbance peak of adenine again to lower wavenumbers (from 1600 to 1570 cm^{-1}) upon base pairing with the oligo(d)T.

Conclusion

Immobilization of thiolated oligonucleotides on silver nanoparticles is a delicate process, requiring a very slow and gradual increase of salt concentration. We found that the success of DNA functionalization strongly depends on the sequence of the oligonucleotides. Unlike in the case of gold nanoparticles, SA appears to produce more stable silver particles than does ST. Analysis of the melting properties of the aggregates in terms of cooperative binding theory points to the lower DNA surface coverage on the Ag/ST than that on the Au/ST particles. The spectral changes upon hybridization of Ag/ST and Ag/SA are also strikingly different from those of gold nanoparticles, exhibiting a marked decrease in intensity of the SPR peak, as opposed to a shift of its maximum. This highly different behavior from that of the gold nanoparticles renders silver nanoparticles to be a valuable candidate as a second marker in DNA hybridization experiments.

The self-assembly of SA and ST on gold film and the subsequent hybridization of their complementary pair, unlabeled or labeled by nanoparticles, monitored by PM-FTIRRAS, allows us to draw two conclusions. First, the adsorption affinity of oligo(d)A to gold surfaces is high enough to compete with the Au−S bond or the hydrogen bonding to complementary bases; however, the highly loaded Au/ST nanoparticles, offering multiple links to the complementary bases, and the attractive force to the gold particle surface are capable of overcoming the nonspecific adsorption of oligo(d)A to the gold film. Second, PM-FTIRRAS is not only capable of detecting the base pairing between DNA strands but also it can distinguish between the types of oligonucleotides (adenine or thymine) attached to the nanoparticles.

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