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Talanta 67 (2005) 456–461
Talanta
www.elsevier.com/locate/talanta

Surface-functionalized silica-coated gold nanoparticles and their bioapplications

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Abstract

Monodisperse Au at SiO_2 nanoparticles has been functionalized with carboxylic groups for further bioconjugation with amino-terminated oligonucleotides. The oligonucleotide-modified Au at SiO_2 nanoprobes have been applied in the fast colorimetric DNA based on the sequence-specific hybridization properties of DNA. Self-assembling behavior of Au at SiO_2 nanoparticles was also investigated.

Keywords: Gold nanoparticles; Silica coating; Surface functionalization; Bioconjugation

1. Introduction

Colloidal functional nanoparticles have been attracting tremendous attention for their promising biological applications, mostly because the synthesis of colloidal nanoparticles is already highly advanced and their surface modification with biological molecules is extensively investigated [1–5]. As an important type of functional nanoparticles, colloidal gold nanoparticles have been used for various biological applications due to their unique size-, composition- and shape-dependent optical properties [1–3], however, the stability and surface functionalization of gold nanoparticles are still problematic in many situations. An ideal solution is to encapsulate them with a protective shell [6,7], and the benefit of silica coating is very obvious in biological applications [8]. In addition to the excellent properties of silica shell with low nonspecific binding and low auto-fluorescence, the highly stable and water-soluble properties of silica-coated gold (Au at SiO_2) nanoparticles make themselves very promising nanomaterials for biolabeling, biosensing, medicinal diagnostics, drug delivery/therapeutics, etc. In this paper, we have successfully prepared monodisperse carboxyl-functionalized Au at SiO_2 nanoparticles followed by the surface bioconjugation via the well-established silica surface chemistry [9]. Also, we have demonstrated the application of bioconjugated Au at SiO_2 nanoparticles with amino-terminated oligonucleotides as bioprobes in DNA detection.

2. Experimental

2.1. Chemicals

$HAuCl_4 \cdot 3H_2O$ (>99.9%, Aldrich), sodium citrate dihydrate (>99%, Aldrich), tetraethylorthosilicate (TEOS, Fluka, ≥99%), 30 wt% ammonia (AR, Mallinckrodt), 3-aminopropyltriethoxysilane (>99%, Merck), succinic anhydride (>99%, Merck), 5-(and-6)-((*N*-(5-aminopentyl)amino)carbonyl) tetramethylrhodamine (A-1318, Molecular Probes), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 99%, Sigma), and sulfo-*N*-hydroxysuccinimide (sulfo-NHS, 98%, Aldrich) were used as received. The oligonucleotides including 5′-TCT-CAA-CTC-GTA-$(CH_2)_7$-NH_2-3′, 5′-H_2N-$(CH_2)_6$-CGC-ATT-CAG-GAT-3′, 5′-TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG-3′, and 5′-TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT-3′ were purchased from Integrated DNA Technologies.

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doi:10.1016/j.talanta.2005.06.020

2.2. Instrumentation

UV–vis spectra were recorded with a Shimadzu UV-1601 spectrophotometer. Fluorescence spectra were obtained on a RF-5301 PC fluorometer. Transmission electron micrographs were measured by a JEOL 1010 transmission electron microscope (TEM) operating at an accelerating voltage of 100 kV. XRD measurements were taken with a Siemens D5005 diffractometer. The atomic concentration of gold colloid was determined by Optima 3000 Perkin-Elmer inductively coupled plasma–atomic emission spectrometry (ICP–AES) using a standard HCl/HNO_3 digestion method. The aggregation and melting analyses were carried out using a HP 8453 diode array spectrophotometer equipped with a HP 89090a Peltier temperature controller.

2.3. Improved silica coating of citrate-stabilized 50 nm gold nanoparticles

Fifty nanometers gold nanoparticles were prepared by a seeded growth of 15 nm gold nanoparticles [10,11]. Twenty milliliters of as-prepared gold colloid was centrifuged at 4000 rpm, and the collected red pellet was re-dispersed into 20 mL deionized water followed by mixing with 100 mL isopropanol in a 250 mL flask under vigorous shaking. After adjusting pH to ~10 by adding 2.5 mL of 30 wt% ammonia solution, 2 mL of 10 mM TEOS isopropanol solution was added within 20 h in an intermissive manner (a time interval of 1 h) followed by further shaking for 12 h. The reaction mixture was then washed with water and ethanol alternatively. The collected Au at SiO_2 nanoparticles were dispersed into 20 mL deionized water ($\sim 6.0 \times 10^{10}$ particles/mL, determined by ICP–AES results) [11]. The Au at SiO_2 nanoparticles with different silica thickness was prepared using different amount of 10 mM TEOS solution. It is noted that reproducible and large-scale production of Au at SiO_2 nanoparticles can be achieved readily.

2.4. COOH-functionalization of Au at SiO_2 nanoparticles

3-(Triethoxylsilylpropylcarbamoyl)butyric acid was prepared according to the reported method [12]. 2.25 mL ethanol solution containing 4.5 mmol of 3-(triethoxylsilylpropylcarbamoyl)butyric acid was mixed with 4 mL aqueous solution containing $\sim 2.5 \times 10^{11}$ Au at SiO_2 nanoparticles and 16 mL ethanol. After shaking for 2 h at room temperature, the reaction mixture was further heated for 1 h at an elevated temperature of 50 °C. After washing with ethanol four times, the collected COOH-functionalized Au at SiO_2 nanoparticles was dispersed into 4 mL deionized water for characterization and bioconjugation.

2.5. Bioconjugation and hybridization of oligonucleotides-modified Au at SiO_2 nanoparticles

Briefly, 100 μL aqueous solution containing $\sim 6.0 \times 10^9$ COOH-functionalized Au at SiO_2 particles were mixed with 10 μL aqueous solution containing 1.1 nmol 5′-TCT-CAA-CTC-GTA-$(CH_2)_7$-NH_2-3′. After adding 5 mg EDC·HCl in 890 μL of 0.1 M sodium phosphate buffer (pH 7.4) and 10 μL of 125 mM sulfo-NHS aqueous solution, the mixture was shaked for 2 h at room temperature. The resulting solution was thoroughly washed with PBS buffer (0.3 M NaCl, 10 mM phosphate of pH 7), the collected oligo-modified Au at SiO_2 nanoparticles (nanoprobe a) were dispersed into 200 μL PBS buffer for hybridization test. Oligo-modified Au at SiO_2 nanoparticles with 5′-H_2N-$(CH_2)_6$-CGC-ATT-CAG-GAT-3′ (nanoprobe b) was also prepared with an identical procedure. A similar hybridization procedure described in our previous report was adopted [11]. The corresponding aggregation process profile of hybridized Au at SiO_2 particles was studied by monitoring the maximum absorption at 550 nm as a function of temperature. The temperature was decreased from 70 to 30 °C at 1 °C interval with a holding time of 1 min at each temperature prior to optical measurement.

3. Results and discussion

Xia and co-workers recently performed a successful silica coating of as-received BBI-50 nm gold particles through the hydrolysis and condensation of tetraethylorthosilicate [13], however, the unknown surface information of commercial gold particles restrained us for further investigations on their surface functionalization and bioconjugation using the well-established silica surface chemistry. It is ideal to directly coat as-prepared citrate-stabilized gold colloids, which are usually very unstable while being transferred into organic solvents like ethanol or isopropanol. In our recent work [11], it was observed that the addition of extra sodium citrates into freshly prepared gold colloids at 100 °C followed by 1 h heating can greatly improved stability of the resulting gold colloids at room temperature, which can be stably transferred into alcohol solution without aggregation. Here we further found that an optimized heat treatment of freshly prepared gold colloids (5–10 min) followed by naturally cooling to room temperature can further increase their stability in alcohol solution. The improved silica-coating procedure can produce gram-level Au at SiO_2 particles. Monodisperse Au at SiO_2 nanoparticles with a uniform silica shell of 70 nm in thickness were successfully prepared, as revealed by the typical transmission electron micrograph in Fig. 1A. After silica coating, an obvious red shift of ~15 nm in the surface plasmon peak of gold nanoparticles ranges from 535 to 550 nm (Fig. 1B) due to an increase in local refractive index of surrounding medium [14]. The X-ray diffraction pattern of Au at SiO_2 nanoparticles (Fig. 1C) shows only the characteristic

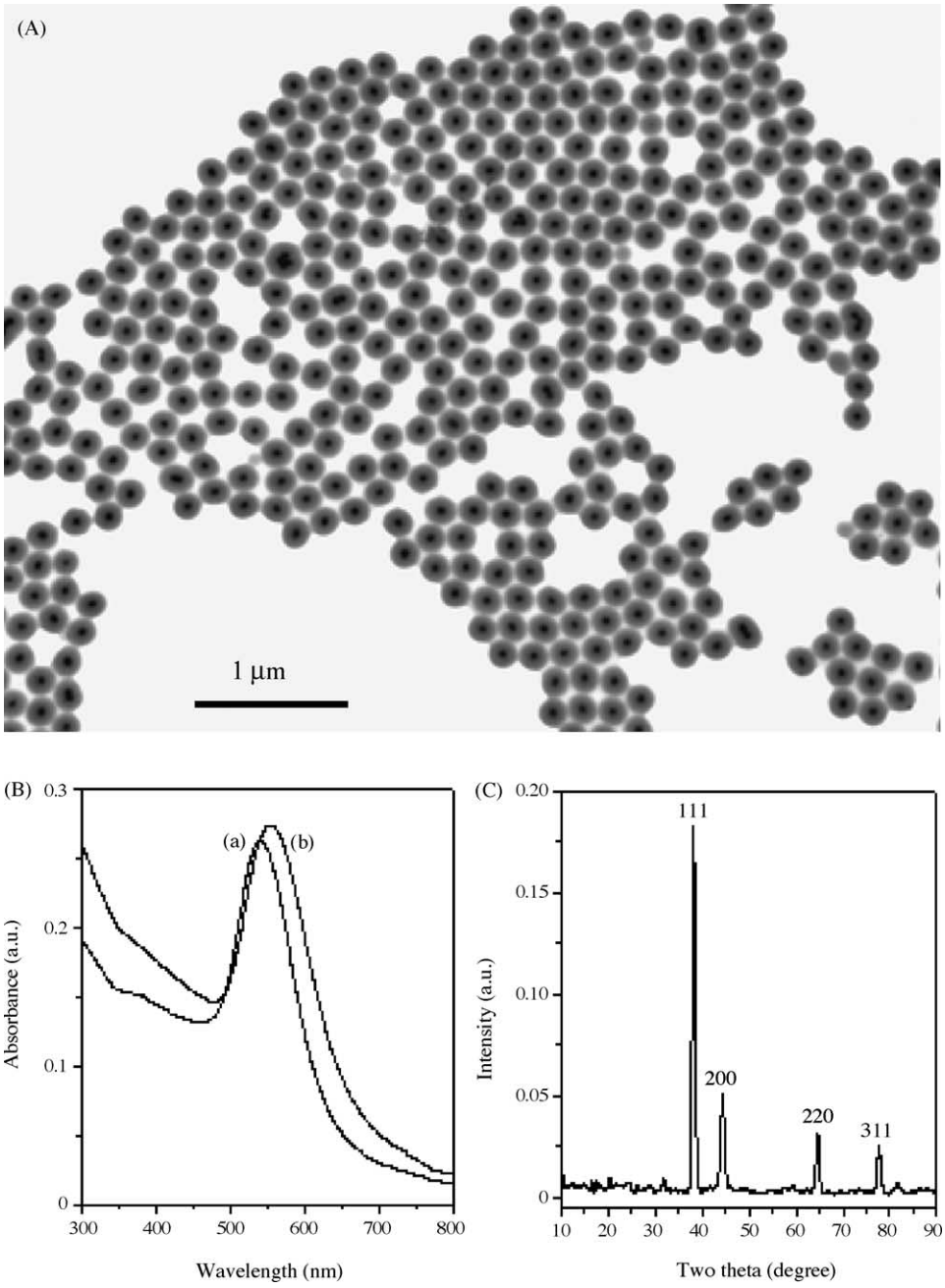

Fig. 1. (A) TEM images of Au at SiO_2 nanoparticles with a silica shell of 70 nm in thickness. (B) UV–vis spectra of: (a) uncoated 50 nm gold nanoparticles, and (b) Au at SiO_2 nanoparticles. (C) XRD pattern of Au at SiO_2 nanoparticles which shows characteristic diffractions of gold at 38.1°, 44.1°, 64.4°, and 78.4°, corresponding to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes, respectively.

diffraction peaks of crystalline Au core [15], indicating the coating of non-crystalline silica. Monodisperse Au at SiO_2 nanoparticles with a different silica thickness of 55 and 35 nm were also prepared by using the different amount of TEOS monomer.

TEM was used to investigate the self-assembly behavior of Au at SiO_2 nanoparticles, which were deposited on the copper grids coated with Formvar film, which is a copolymers formed from polyvinyl alcohol and formaldehyde with polyvinyl acetate. When dispersing a drop of lowly concentrated Au at SiO_2 nanoparticles on a copper grid overnight, one line of Au at SiO_2 nanoparticles can self assemble along the edge of broken Formvar film (Fig. 2A). Interestingly, a second layer of Au at SiO_2 nanoparticles can further self assemble on the first layer of Au at SiO_2 nanoparticles (Fig. 2B). So we believe that the hydrogen bonding between OH-rich surfaces of Au at SiO_2 nanoparticles rather than repulsive force between negatively charged

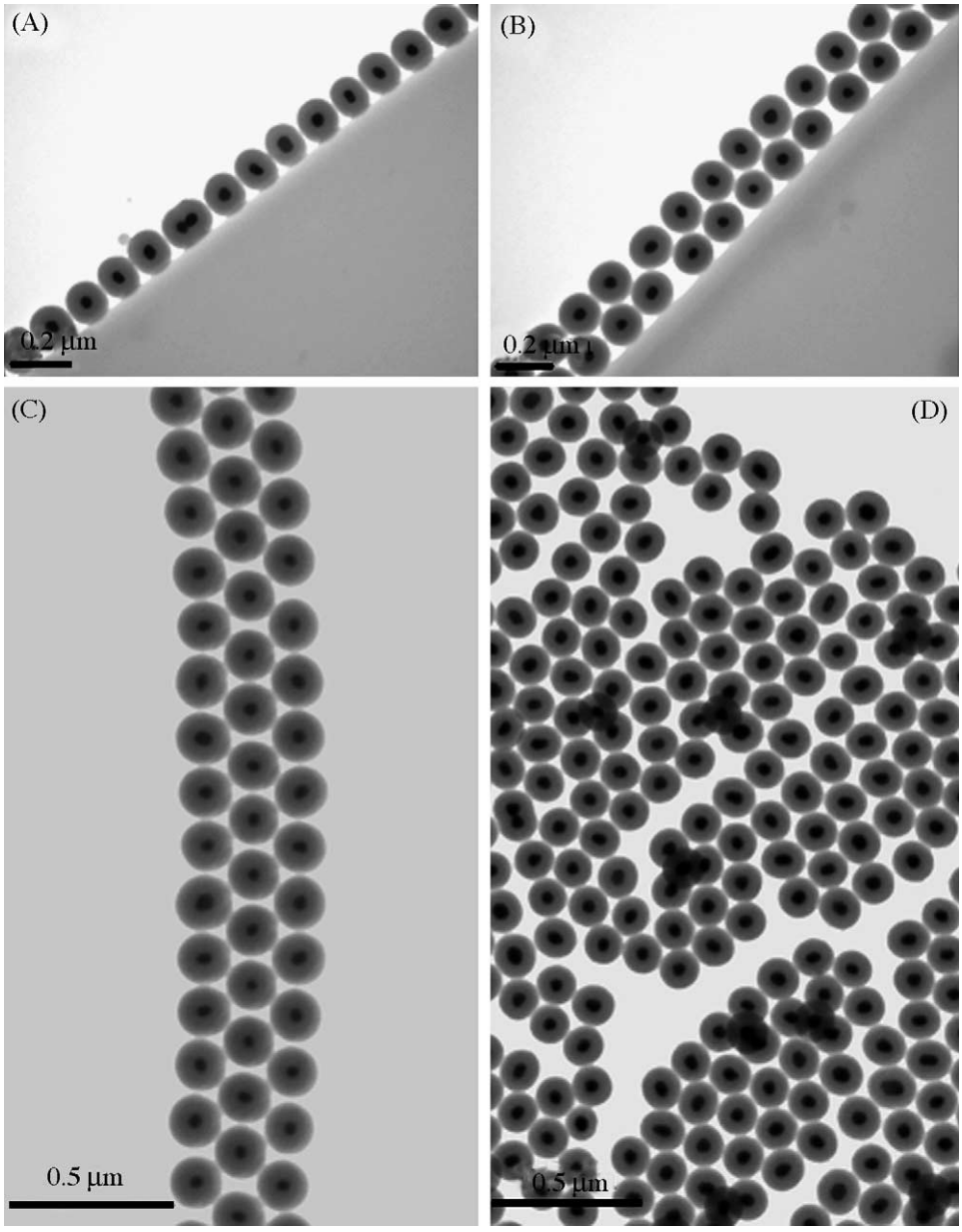

Fig. 2. (A–D) Self-assembling behavior of Au at SiO_2 nanoparticles along the edge of Formvar film or on the top surface of Formvar film.

Au at SiO_2 nanoparticles could play an important role in the self-assembly process. Meanwhile, this is also supported from the ordered array of Au at SiO_2 nanoparticles on the surface of Formvar film (Fig. 2C). The Au at SiO_2 nanoparticles would also like to form a second layer to more closely interact silica particles instead of finding a place to stay in the first layer (Fig. 2D) due to the strong interaction between silica spheres. All these results indicate that the as-prepared Au at SiO_2 nanoparticles can be used not only for bioconjugation in biomedical applications but also for spontaneous particles self-assembling in diverse applications ranging from photonic crystals to surface nanopatterning.

Aldehyde-functionalized Au at SiO_2 nanoparticles were reported previously [11]. Herein monodisperse Au at SiO_2 nanoparticles have been covalently grafted with carboxylic groups using a silane coupling agent, i.e. 3-(triethoxylsilylpropylcarbamoyl)butyric acid. Carbodiimide coupling reagents (EDC/sulfo-NHS) was used to determine the density of surface-grafted carboxylic groups [16]. 0.2 mL aqueous solution containing $\sim 1.2 \times 10^{10}$ Au at SiO_2 nanoparticles was mixed with 0.1 mL of 0.58 mM A-1318 ethanol solution, 10 mg EDC·HCl, and 20 μL of 125 mM sulfo-NHS aqueous solution followed by shaking for 2 h. After thorough washing with ethanol four times, the collected sample was dispersed into 1 mL ethanol for fluo-

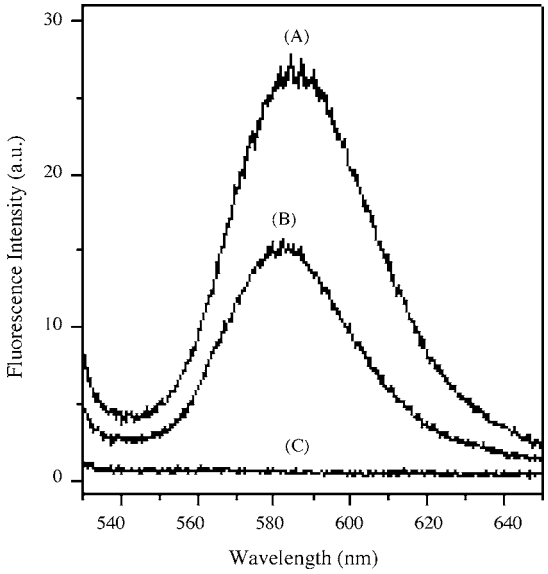

Fig. 3. Fluorescence spectra of: (A) A-1318 reacted with 3-(triethoxylsilylpropylcarbamoyl)butyric acid as a reference; (B) COOH-modified Au at SiO_2 particles reacted with A1318, and (C) bare Au at SiO_2 particles as a control after the physical adsorption of A-1318 was washed thoroughly.

rescence measurement ($\lambda_{ex} = 520$ nm). As compared to the reference sample of A-1318 in ethanol after reacted with 3-(triethoxylsilylpropylcarbamoyl)butyric acid, the grafting level of carboxyl groups is determined to be $\sim 1.8 \times 10^4$ carboxylic groups per Au at SiO_2 particle (Fig. 3A and B) [16]. The blank experiment with bare Au at SiO_2 particles showed there is no detectable physically adsorbed A-1318 dye molecules on Au at SiO_2 particles because silica has unique properties of low nonspecific binding (Fig. 3C).

Surface-grafted carboxyl groups on Au at SiO_2 particles can provide a specific platform for the covalent attachment of biomolecules such as amino-rich proteins or amino-terminated oligonucleotides. The bioconjugation of COOH-functionalized Au at SiO_2 nanoparticles with amino-terminated oligonucleotides was done by the EDC/sulfo-NHS chemistry [16]. Hybridization of oligonucleotides-modified Au at SiO_2 nanoparticles was done with a similar procedure reported in our recent publication [11]. The as-prepared two nanoprobes (a and b) in 200 μL PBS buffer, respectively, were mixed followed by heating up to ~70 °C for 10 min. After being cooled down to room temperature, there were no obvious aggregates formed in the solution. The two as-prepared nanoprobes (a and b) in 400 μL PBS buffer were mixed with 100 μL of 1.3 nmol complementary target oligonucleotide of 5′-TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG-3′ together followed by heating up to ~70 °C for 10 min. During the process to cool the solution, there were obvious macroscopic red-color aggregates formed in solution in a short time of less than 3 min. Au at SiO_2 nanoprobe samples before and after the addition of complementary target

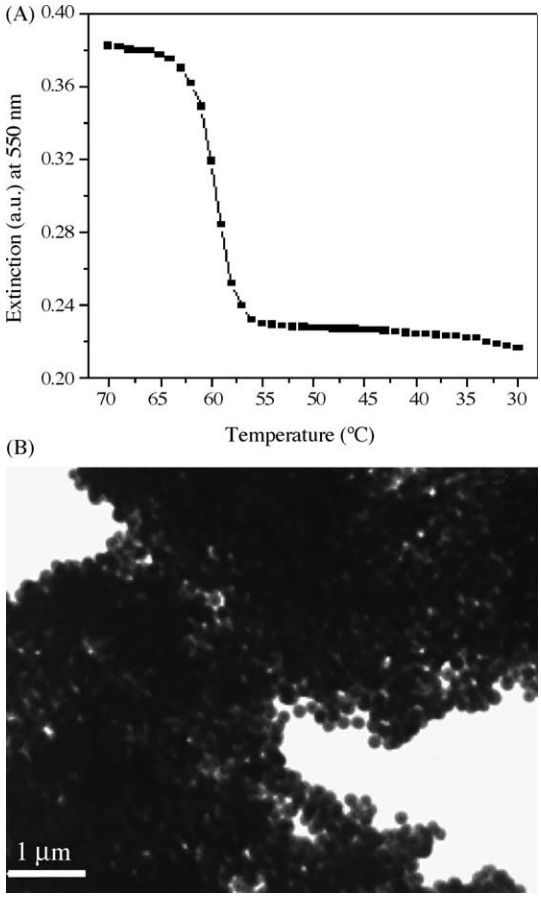

Fig. 4. (A) Aggregation profile of oligo-modified Au at SiO_2 nanoparticles as a function of temperature. (B) TEM image of Au at SiO_2 aggregates formed by the DNA hybridization between the two oligo-modified nanoprobes and the complementary target oligonucleotides.

oligonucleotide were studied with TEM. Control experiment using non-complementary target oligonucleotide of 5′-TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT-3′ showed that there were no macroscopic red-color aggregates formed.

The aggregation process of self-assembled oligo-modified Au at SiO_2 nanoparticles as a function of temperature was monitored through the absorption intensity change of gold nanoparticles at the maximum wavelength of 550 nm. The solution temperature was decreased from 70 to 30 °C at 1 °C intervals with a holding time of 1 min at each point prior to spectroscopic measurement. The absorption intensity was remained almost unchanged when the temperature decreased from 70 to 63 °C. However, with the further decrease of temperature from 62 to 57 °C, there was a sharp drop in absorption intensity due to the fast hybridization/aggregation of the two Au at SiO_2 nanoprobes with the complementary target oligonucleotides. The absorption intensity became stable again when the solution temperature was further lowered from 55 °C to room temperature. As compared with the aggregation process of oligo-modified gold nanoparticles as reported by other workers [17], there is no similar

color change from red to purple for our samples before and after hybridization. The TEM image clearly showed large aggregates consisting of a large number of Au at SiO_2 nanoparticles (Fig. 4B). However, there is no observable red shift of absorption peak position because the hybridization of oligo-modified Au at SiO_2 nanoprobes did not result in very close proximity of gold nanoparticles due to the comparatively thick silica shell. Similar to our previous work about CHO-functionalized Au at SiO_2 nanoparticles [11], the red macroscopic Au at SiO_2 aggregates resulting from COOH-functionalized Au at SiO_2 nanoparticles in this work can also be melted at an elevated temperatures above melting point, resulting in similar reversible aggregation and melting behavior (Fig. 4A).

4. Conclusion

We have successfully prepared monodisperse COOH-functionalized Au at SiO_2 nanoparticles, which have been further covalently conjugated with amino-terminated oligonucleotides. The oligo-functionalized Au at SiO_2 nanoparticles are very useful as robust probes for the fast colorimetric DNA detection based on the sequence-specific hybridization properties of DNA. Simple diagnostic process can be achieved by simply heating the solution of DNA-attached Au at SiO_2 nanoparticles to a temperature higher than melting point, and the following cooling process can lead to the obvious aggregates/precipitates for the fast colorimetric DNA detection.

Acknowledgements

This work was supported by the National University of Singapore and the Institute of Materials Research and Engineering.

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