A Nanoparticle-Based Solution DNA Sandwich Assay Using ICP-AES for Readout

Linlin Wu,[†] Liwen Qiu,[‡] Chunsheng Shi,[†] and Jin Zhu*,[†]

Department of Polymer Science and Engineering, State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, and State Key Laboratory of Mineral Deposit Research, Nanjing University, Nanjing 210093, China

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We report herein a nanoparticle-based methodology for detecting DNA in solution using inductively coupled plasma atomic emission spectrometry (ICP-AES) as a readout tool. This represents the first homogeneous solution assay of biologically significant targets by employing ICP-related techniques. Two types of particles are employed: silica nanoparticles or gold nanoparticles functionalized with oligonucleotides that are capable of hybridizing with half of the target DNA sequence as signal readout components, and magnetic microparticles functionalized with oligonucleotides that are capable of hybridizing with the other half of the sequence as capture components. In the presence of target DNA, three components form typical sandwich structures, and the application of a magnetic field could effectively separate them from the rest of the solution. Subsequent application of ICP-AES effectively provides an inorganic elemental readout for the diagnosis of target DNA.

Introduction

The past decade has witnessed significant progress in the controlled synthesis of nanoparticles (NPs) in a monodisperse format with varied shapes, sizes, and compositions. Methods for preparing and handling NP−biomolecule conjugates have also advanced to a stage where stable probes could be fabricated on a routine basis.[1] These developments have contributed to the emerging exploitation of NPs in molecular diagnostics and biomedical research, in many ways complementing the traditional methods. DNA detection is central to the diagnosis of genetic and pathogenic diseases and has benefited tremendously from the advances. Thus far, detection methods employing NPs as probes, such as colorimetric,[2−5] fluorescence,[6−9] resonant light-scattering,[10,11] scanometric,[12] and Raman spectroscopy,[13] have been based mostly on the unique physical properties associated with NPs themselves.[14] These strategies have afforded highly selective and sensitive DNA assays that are derived from superior properties associated with the NP-labeled DNA (e.g., sharp melting transitions and signal amplification through bio-barcoding). However, challenges remain: for example, many of these methods suffer from limited multiplexing capabilities due to a limited choice of physical signatures, albeit a combination of NPs with distinct properties could occasionally circumvent the problem. Chemical signatures, particularly elemental compositions, offer a potentially attractive alternative to DNA detection by providing a direct, multiplexable in principle, facile readout. Herein we report a solution DNA sandwich assay based on oligonucleotide-functionalized NP probes using inductively coupled plasma atomic emission spectrometry (ICP-AES) as the readout platform.

Prior to this work, the Wang group used inorganic nanocrystal tracers as electrochemical tags, by carrying out stripping voltammetry on the heavy metals, for the detection of DNA.[15,16] One limitation is that only a selected number of elements could be utilized for simultaneous coding purposes. In contrast, ICP is a well-established atomization, ionization, and excitation source that is used in combination with either AES or mass spectrometric (MS) detection for trace elemental analysis. ICP has a much higher resolution in principle, allowing a vast majority of elements to be detected in a sequential and simultaneous mode. The Zhang[17] and Baranov[18] groups developed an immunological method for the detection of proteins based on ICP-MS. The Merkoçi group used ICP-MS for the immunoassay of peptide-conjugated DNA,[19] which required a proper peptide sequence that was covalently linked with DNA and the use of antibodies. Moreover, their methods were invariably based on the capture of NP probes onto a planar substrate, either a nitrocellulose membrane or a 96-well microtiter plate.

ICP-AES is one of the most commonly employed techniques for elemental analysis. Its high specificity, multi-element capability and good detection limits result in the use of the technique in a large variety of applications. The instrument measures characteristic emission spectra by optical spectrometry. A plasma source is used to dissociate the sample into its constituent atoms or ions, exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength depending on the element that is present. This light is recorded by an optical spectrometer, and the technique provides a quantitative analysis of the original sample by calibrating against standards. ICP-AES remains a competitive analytical tool in certain aspects compared with ICP-MS. For example, much more standard methods are adopted by ICP-AES, and high spectral resolution helps eliminate interelement interference. Further, mass effect and isotope interference do not exist in the ICP-AES system. This analytical tool thus far hasn't been exploited in the DNA detection arena. Silica NPs have many advantages by being biocompatible and possessing well-developed surface modification chemistry and water dispersibility, and gold NPs (Au NPs) are also well

* Corresponding author. Phone: +86 25-8368-6291. Fax: +86 25-8331-7761. E-mail: jinz@nju.edu.cn.

[†] Department of Polymer Science and Engineering, State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering.

[‡] State Key Laboratory of Mineral Deposit Research.

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Scheme 1. Sandwich-Type Assay for DNA Detection

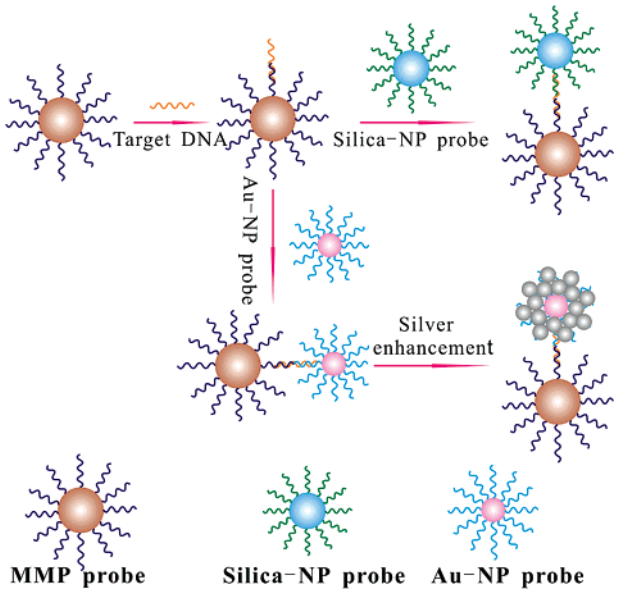

Table 1. Operating Conditions of JY 38 S ICP-AES

radio frequency powder	900 W
operating frequency	40.68 MHz
focal length	1 m
grating	4320 grooves mm^{-1}
practical resolution ratio	0.005 nm
coolant argon flow	12 L min^{-1}
auxiliary argon flow	0.3 L min^{-1}
coating gas	0.2 L min^{-1}
wavelengths range	165−800 nm
wavelengths for Si	251.611 nm
wavelengths for Au	242.795 nm
detection limits	0.01−0.1 μg mL^{-1}
RSD	≤2%

established in a variety of bioconjugates. Therefore, we chose silica NPs and Au NPs, two of the representative NPs, as probes to evaluate the feasibility of detecting target DNA in a solution format. Amino-modified magnetic microparticles (MMPs) functionalized with DNA were utilized to capture and separate target DNA, which then underwent further hybridization with silica- or Au-NP probes in solution (Scheme 1). The captured NP probes were then subjected to ICP-AES for elemental readout. Our solution assay offers, in principle, faster target-binding kinetics than the planar substrate format because of the homogeneous nature of the target-capture procedure.[20]

Experimental Section

Instrumentation. A mono-channel scanning ICP-AES (model JY 38 S, France) was used for this experiment. The optimal operating parameters for the instrument are summarized in Table 1. The detection limits of this apparatus are 0.003 μg/mL for silicon element, and 0.001 μg/mL for gold element. For detection values in micrograms per gram, we assume that one gram of the solution equals one milliliter. Prior to the analysis of Si, solutions of calibration verification standards, containing 0, 3, 6, and 10 μg/g Si were used for the calibration and to establish a linear relationship. For Au, solutions containing 0, 1, 2, and 5 μg/g Au were used. Time-resolved Fourier transform infrared (FT-IR) spectroscopy was recorded on a Bruker IF 66/S facility, and ultraviolet−visible−near-infrared (UV−vis−NIR) extinction spectroscopy measurement was carried out on a Perkin-Elmer Lambda 35 instrument. Transmission electron microscopy (TEM) was performed on either a JEOL JEM-1230 or JEOL JEM-200CX facility.

Table 2. Oligonucleotide Sequences and Modification

target DNA1	5′-GGATTATTGTTAAATATTGATAAGGAT-3′
capture DNA2	5′-ATTTAACAATAATCCAAAAAAAAAA-SH-3′
capture DNA3	5′-HS-AAAAAAAAAAATCCTTATCAAT-3′
random DNA4	5′-TAGGAATAGTTATAAATTGTTATTAGG-3′
passivation DNA5	5′-HS-AAAAAAAAAA-3′

Materials. *m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS), and succinimidyl 4-[*p*-maleimidophenyl] butyrate (SMPB) were obtained from Pierce Biotechnology Co. Tetraethyl orthosilicate (TEOS, 98%), 3-aminopropyltrimethoxysilane (APTMS, 97%), and silver enhancement solution were purchased from Aldrich Chemical Co. Sodium phosphate dibasic anhydrous (Na_2HPO4, traceselect ⩾ 99.99%), sodium dihydrogen phosphate anhydrous (NaH_2PO4, traceselect ⩾ 99.0%), and sodium chloride (NaCl, traceselect ⩾ 99.99%) were from Fluka Chemical Co. Amino-functionalized MMP solution (Dynal Biotech Co., Dynabeads M-270 Amine) was commercially available from Invitrogen Co. $HAuCl_4 \cdot 4H_2O$ (99.9%) was from ShangJuly Chemical Co., Ltd. All oligonucleotides (Table 2) were synthesized and purified by Sangon, Inc. (Shanghai, China), and their concentrations were quantified by optical density (OD) at 260 nm. Capture strands DNA2 and DNA3 are thiolated oligonucleotides with an $-(A)_{10}-$ spacer at the 3′ or 5′ end, respectively, which complement half of the target sequence of DNA1. DNA4 is a random sequence, which is not cognate to either DNA2 or DNA3. DNA5 is a thiolated DNA for passivating particle surfaces. Nanopure water (18.2 MΩ·cm) purified by a Sartorius Arium 611 system was used throughout the experiment.

Preparation of Modified Silica NPs. First, ~400-nm monodisperse silica NPs were prepared by the Stöber method.[21] Briefly, 50 mL of 0.9 M TEOS in absolute ethanol was added dropwise to a solution consisting of 100 mL of absolute ethanol and 44 mL of ammonia (28−30%) under vigorous stirring. The reaction was allowed to continue for 12 h at room temperature. Second, silanization of the silica NPs with APTMS was performed. To achieve this, 500 μL of APTMS was injected into the first reaction vessel, and the mixed solution was stirred for 12 h. The resulting APTMS-modified silica NPs were washed six times with ethanol by centrifugation, decanting and redispersing in ethanol. Afterward, the powder was dried overnight at 100 °C.[22] Third, the NPs were modified with an MBS cross-linker.[23] Anhydrous acetonitrile (2 mL) containing 0.00086 g of MBS cross-linker was added to 10 mg of APTMS-modified silica. The reaction was allowed to proceed for 1 h at room temperature under constant vortex and then rotate for 11 h. Finally, the particles were centrifuged, and the supernatant was decanted, washed twice with acetonitrile, washed once with coupling phosphate-buffered saline (PBS) buffer 1 (1 M NaCl, 10 mM sodium phosphate, pH 7.0), and dried overnight at 50 °C (MBS−APTMS−silica).

Silica-NP Probes. Target-binding DNA1-modified silica-NP probes were synthesized by adding 1 OD of DNA2 to a solution containing 2.3 mg of MBS−APTMS−silica in coupling PBS buffer 1, and the final concentration of the DNA2 was 3.4 μM. The reaction between the maleimide group and the -SH group of the DNA2 was allowed to proceed for 10 h under rotation. After reaction, the particles were centrifuged and decanted, followed by washing three times with coupling PBS buffer 1. The excess maleimide groups were passivated with 1 mL of 7.2 μM thiolated-A10 DNA5 in coupling PBS buffer 1. The resulting particles were washed with coupling PBS buffer 1 three times and finally redispersed in 2 mL of PBS buffer 2 (0.2 M NaCl, 10 mM phosphate buffer, pH 7.2). We estimated the DNA density by measuring the decrease in the absorbance of supernatant at 260 nm after the modification of particles, which was used to calculate the total amount of DNA that was loaded onto the particle surface. There are, on average, 5.7×10^3 DNA2 strands per silica NP.

Au-NP Probes (13 nm). Au NPs with an average diameter of 13 nm were synthesized by the citrate reduction of $HAuCl_4$.[24] Au-NP

probes were prepared according to a slightly modified procedure in the literature.[2] Briefly, 100 μL of 34 μM thiolated target-binding DNA2 was incubated in 2 mL of 13 nm Au-NP solution (~17 nM). After standing for 16 h, the solution was brought to 0.1 M NaCl, 10 mM phosphate buffer (pH 7), in a stepwise manner with 0.3 M NaCl, 30 mM phosphate buffer (pH 7), and allowed to age for 40 h. Excess reagents were removed by centrifugation at 14 000 rpm for 25 min. Finally, the red oily precipitate was washed by centrifugation and then redispersed in 2 mL of buffer (0.2 M NaCl, 0.01% tween 20, 10 mM phosphate buffer, pH 7.2).

MMP Probes. Amino-functionalized MMPs (2.8 μm, with polystyrene coating) were modified with 5′-thiol-terminated DNA3 using a method developed by the Mirkin group.[25] The process is described as follows: MMPs (300 μL, 30 mg mL^{-1}) were washed three times with 300 μL of anhydrous dimethyl sulfoxide (DMSO), and then redispersed in a solution of SMPB (1 mg) in anhydrous DMSO (100 μL). The suspension sealed with foil and Parafilm was allowed to vortex for 30 min and rotate for another 12 h at room temperature. After reaction, the beads were magnetically separated and washed three times with anhydrous DMSO (300 μL) and then twice with coupling PBS buffer 3 (0.15 M NaCl, 0.1 M sodium phosphate, pH 7.0). 5′-Thiol-terminated DNA3 (100 μL, 39 mM) was then added to the MMP wet cake. After reaction for 10 h under rotation, the MMPs were washed three times with coupling buffer 3. The resulting MMPs were passivated with 100 μL of 72 μM thiolated-A10 DNA in coupling buffer 3 for 10 h and washed three times with PBS buffer 2. Finally, the DNA3- and DNA5-modified MMPs were redispersed in 2 mL of assay buffer (0.2 M NaCl, 0.1% tween 20, 10 mM phosphate buffer, pH 7.2), and stored at 4 °C. There are, on average, 2.685×10^5 DNA3 strands per particle.

Detection with Silica-NP Probes. The assay was conducted by a typical procedure of three-component sandwich assay format. Three methods—direct sonication (1), acid dissolution (2), and high-temperature release (3)—were employed in this experiment before the solution of MMPs with target-linked silica NPs was introduced into the ICP system. Initially, 30 μL of target DNA1 solution was added to MMP probes (50 μL). The mixture was warmed to 45 °C and allowed to stay for 30 min, then rotated for 3 h at 25 °C. After the reaction was completed, the MMP−target complexes were separated on a 12-well-plate magnet separator (Promega Co.) and washed three times with the assay buffer. The resulting MMP−target complexes were redispersed in the 50 μL of assay buffer, followed by the addition of 50 μL of silica-NP solution. After hybridization for 2 h, the system was washed six times with assay buffer as described before. In method 1, the final washed system was redispersed in 1 mL of nanopure water by sonication, followed by introduction into ICP. In method 2, before introduction into ICP, 100 μL of hydrochloric acid was added to the final washed system in order to dissolve the MMPs, followed by dilution to 1 mL with nanopure water. In method 3, the final washed system was redispersed in 200 μL of nanopure water, followed by heating at 80 °C for 5 min, and then sonicated at 80 °C for 10 min to ensure effective release of the silica NP. Before measuring the signal of silicon, removal of all of the MMPs by a magnetic separator from solution was performed. This high-temperature release step was repeated two more times to completely collect the silica NPs that were linked to MMPs through target DNA. The whole solution (600 μL) of released silica NPs was diluted to 1 mL by nanopure water for detection.

Detection with Au-NP Probes. The procedure for detecting target DNA1 in solution by Au-NP probes was similar to that by silica-NP probes. After completing the preparation of MMP−target complexes as well as washing three times with assay buffer, 50 μL of Au-NP probes was added to the above MMP−target conjugates, which were redispersed in 50 μL of assay buffer. The mixture was hybridized for 2 h under rotation at room temperature, followed by washing six times with phosphate-buffered nitrate (PBN) buffer (0.3 M $NaNO_3$, 10 mM phosphate buffer, pH 7.0). Silver enhancement solution was used in this experiment to compare the results of the assay by color discrimination. The final washed system was exposed to 200 μL of silver enhancement solution (100 μL of silver enhancement solution A and 100 μL of silver enhancement solution B), followed by vortex for 2 min. Then the unreacted silver enhancement solution was removed by placing the reaction vessel on a magnet separator for 1 min. Finally, before introduction into the ICP, the resulting system was washed six times with 0.3 M PBN and dissolved by 1 mL of 0.1% aqua regia solution (prepared by mixing concentrated nitric acid and concentrated hydrochloric acid in a volumetric ratio of 1:3) in nanopure water.

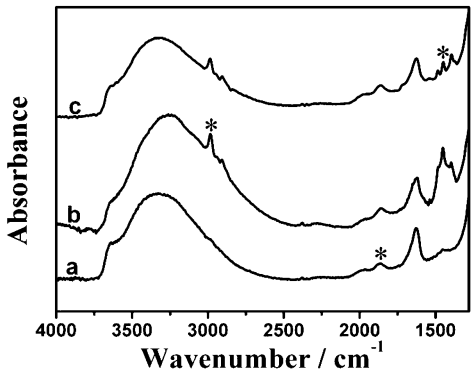

Figure 1. IR spectra of silica NPs after each modification step: (a) neat silica NPs, (b) after modification with APTMS, and (c) after further modification with MBS.

Results and Discussion

Preparation of Modified Silica NPs. Silica NPs were employed as probes in this typical sandwich-type experiment. Silica NPs were chosen for the initial proof-of-concept demonstration here because they are accessible in macroscopic quantities and in monodisperse form. Moreover, methods exist for facile incorporation of other types of NPs and therefore chemical coding elements into silica NPs.[26] To prepare the probes, amino groups were first introduced onto the surface of silica NPs with APTMS. The APTMS-functionalized silica NPs were then activated with a heterobifunctional cross-linker (MBS) that contains *N*-hydroxysuccinimide ester and maleimide groups. The various heterobifunctional cross-linkers that are adopted in the surface modification perform differently in different chemical environments and substrate surfaces. No single cross-linker excels in all aspects. As a result, the choice of a cross-linker for a particular substrate is largely a trial-and-error process. We chose MBS for the modification of silica NPs by following an optimized method developed by Jin et al.[23] Silica NPs after this step possessed maleimide-exposed chain ends for further modification with the sulfhydryl groups on thiolated oligonucleotides to form stable thioether bonds. The modification of silica NPs with APTMS has been reported,[23] wherein an anhydrous toluene solution of APTMS was reacted with the as-prepared silica NPs. However, no IR signatures associated with $-CH_2$ were observed. As a result, we modified the procedure by directly adding APTMS to the Stöber system, in the presence of ammonia and ethanol. The IR spectra of modified silica NPs before the loading of oligonucleotides are shown in Figure 1. The signature peaks marked by the asterisks in the IR spectra compared well with those in the literature, indicating successful modification with APTMS and MBS.[23] In addition, the control experiment carried out in the absence of APTMS did not yield silica NPs with a $-CH_2$ IR signature, confirming that $-CH_2$ came from surface-anchored APTMS instead of incompletely hydrolyzed TEOS.

Assay with Silica-NP Probes. The reason 400-nm silica NPs were chosen is based on the following considerations: although

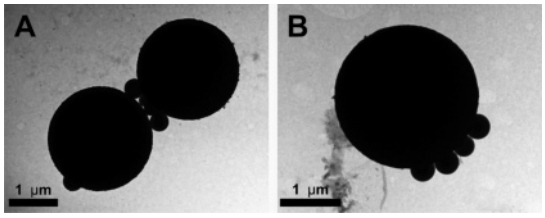

Figure 2. TEM images of the sandwich structure formed by silica NPs and MMPs linked with DNA1.

silica NPs of smaller diameters exhibit better dispersibility, they could only allow limited signal amplification, while larger silica NPs would result in poor dispersibility and therefore poor binding kinetics. In our assay system, we utilized MMP probes as a convenient handle for facile separation of target DNA and, further, capturing silica NPs for the ICP-AES readout. A DNA sequence, DNA1, which is associated with the anthrax lethal factor, was chosen as the target for the first set of experiments.[27] To evaluate the feasibility of this assay format, we first investigate the formation of a sandwich structure comprised of MMPs and silica NPs by TEM. Consistent with our expectation, in the presence of target DNA, silica NPs formed satellite-like structures surrounding MMPs in solution (Figure 2). The nonuniform coating of silica NPs on the surface of an MMP is largely due to the less favorable binding kinetics derived from two factors: (1) the large size of silica NPs leads to less prominent Brownian motion and correspondingly less collision probability with MMPs; and (2) the loading density of oligonucleotides on silica NPs is 1.9 pmol/cm^2, a value that is an order of magnitude lower than that on Au NPs (19 pmol/cm^2 for oligonucleotides with an $-(A)_{10}-$ spacer on 15 nm Au NPs),[28] whereas the total surface area of silica NPs is comparable to that of Au NPs in a given amount of buffer solution. This leads to the less hybridization probability. The above two factors contribute to the formation of a lower amount of sandwich structures and nonuniform coating of silica-NPs on the surface of an MMP.

The three-component sandwich structures exist either as bridged networks (Figure 2A) or discrete MMPs (Figure 2B). The networked structures were not observed in the assembly with Au-NP probes.[29] This phenomenon most likely results from the size of the silica NPs, which is much closer to that of MMPs compared with Au NPs. The sub-micrometer-sized silica NPs will obviously result in different hybridization kinetics than Au NPs and require screening of experimental parameters that favor their binding to micrometer-sized MMPs. Nevertheless, even though the hybridization efficiency and therefore the captured silica NPs are not yet optimized, they have allowed us to demonstrate the ICP-AES-based solution DNA sandwich assay with this system. Because NPs in ICP have the same atomization efficiency as that of ion solutions,[17] elemental signals could be obtained by subjecting solution silica NPs to the ICP-AES facility for analysis. Our own experiment confirmed that the silicon signals of solution NPs could be directly measured by ICP-AES. A slight amount of impurity from the buffer solution could significantly increase the background signals for designed target elements. Therefore, salts with stringent traceselect values were employed in the preparation solutions. We examined target DNA concentration-dependent responses by three different methods. These methods differ in the pretreatment of MMP−NP conjugates before the ICP-AES measurement. Method 1 relied on direct sonication to disperse the MMP−NP complexes in solution in situ. Method 2 used acid to dissolve MMPs to yield a silica-NP solution. Method 3 took advantage of the high-temperature release of the captured silica NPs that resulted from DNA dehybridization at this temperature. MMPs are extremely stable, even at high temperatures, as evidenced by TEM. This ensures the integrity and reproducibility of the high-temperature release method. A series of target DNA1 concentrations were studied, ranging from 3.5 μM to 3.5 pM, and, simultaneously, DNA4 (3.5 μM) with a noncomplementary, random sequence was used as a control to preliminarily test the selectivity of our system. From Figure 3, several observations deserve comments: first, the silicon signals in the presence of DNA4 were invariably at the background level (no target), irrespective of the methods adopted. Second, a positive correlation between the silicon signal and the target DNA concentration, especially at high concentrations, was identified for all three methods. The experiments presented for silica NPs, repeated on separate batches of samples, confirmed the reproducibility of the results in Figure 3 and the reliability of the protocols. Third, method 1 displayed higher signals at high DNA1 concentrations compared those of with methods 2 and 3, most likely because the ICP-AES values obtained reflected the full amount of hybridized silica NPs. The reason the acid dissolution method gave a lower signal might be due to the interference of the polystyrene coating after the dissolution of MMPs. High-temperature thermal release, even repeated three times, could not ensure complete removal of all the silica NPs, as evidenced by TEM. The signal generated from the sample of 3.5 pM is higher compared with that from the sample of 350 pM (Figure 3C). The reason why this happens is not clear at this point, but is conjectured to be due to less efficient thermal release for a

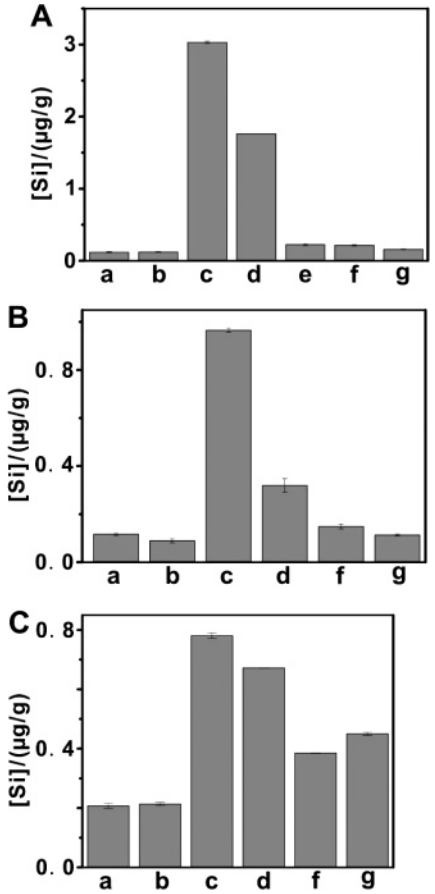

Figure 3. Silicon signal obtained by ICP-AES with method 1 (A), method 2 (B), and method 3 (C) in a series of DNA concentrations: (a) 0, (b) 3.5 μM DNA4, (c) 3.5 μM DNA1, (d) 35 nM DNA1, (e) 3.5 nM DNA1, (f) 350 pM DNA1, and (g) 3.5 pM DNA1.

higher DNA concentration and, correspondingly, a higher number of DNA interlinks between silica NPs and MMPs under the experimental conditions employed herein. Finally, even with the target DNA concentration as low as 3.5 pM, the silicon signal (0.16 μg/g) was higher than the background (0.12 μg/g), suggesting that our assay system could get down to this concentration range. It is important to note that silica-NP probes were always freshly prepared immediately prior to use, and, under this condition, repeat experiments verified the reproducibility of our detection data. However, silica-NP probes might degrade over time and affect the overall performance of our assay system after being left standing for an extended period of time. Indeed, the performance of our assay system is less satisfactory after one week.[30] In an ideal situation, if the surface of MMPs is completely covered with silica NPs and all the silica NPs are detected by the ICP-AES facility, a lower detection limit can be achieved. This requires substantial optimization of the experimental variables, which is currently underway in our laboratory.

Assay with Au−NP Probes. We first examined the colorimetric method to compare our system with the scanometric assay carried out earlier by the Mirkin group.[12] Prior to our investigation, the silver enhancement method had also been used for electrochemical detection in a homogeneous solution on particle assembly directed by a biotin−streptavidin pair and oligonucleotides.[31,32] However, no macroscopic outlook was presented for the resulting silver-enhanced nanostructures. The principle of silver enhancement is that, in the presence of Au NPs, silver ions are reduced by hydroquinone to yield silver metal on the surface of Au NPs. Herein, the silver enhancement with Au NPs in a homogeneous solution was explored in a similar fashion. Similar to what was observed on a flat surface, when we applied silver enhancement to the sandwiched MMP−NP complexes in solution, the macroscopic outlook of the sample turned black. This promising result may broaden the application of silver enhancement in direct homogeneous solution assay systems. In addition, before the application of silver enhancement solution, while a colloidal solution of Au NPs with diameters of 13 nm exhibited a red color, in a typical sandwich assay, after hybridizing with MMPs in the presence of target DNA1 of higher concentrations (3.5 μM and 35 nM), the solution color faded when the reaction vessels were placed on a magnetic separator. This reflected the fact that most of the Au NPs were captured on MMPs. However, at lower target DNA concentrations (350 pM and 3.5 pM), the color of the leftover solution could not be differentiated from the background. In contrast, the silver enhancement allowed the signal to be amplified, and, even at target DNA concentrations as low as 3.5 pM, the silver-enhanced sample displayed a darker color than the background (Figure 4). Thus, the color of the MMP−NP complexes obtained after silver enhancement indirectly reflected the degree of hybridization in different concentrations of the target DNA in solution. Several experimental conditions employed herein deserve comments: we chose PBN buffer to exchange and replace PBS buffer to avoid a direct precipitation reaction between residual Cl^- and Ag^+ and loss of silver enhancement on MMP−NP complexes. The duration of silver enhancement significantly impacted the color of the resulting sample. The sample apparently turned black after application of the enhancement solution for 2 min. This optimum condition was adopted throughout the experiment, by being capable of signal amplification and differentiation against background.

Silver-enhanced MMPs continued to have a good dispersibility in solution. The fact that 13 nm Au NPs were too small

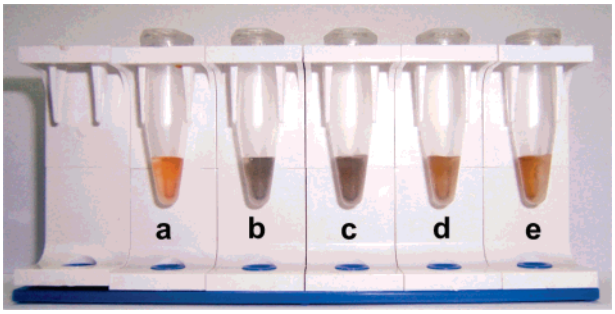

Figure 4. Images of silver-enhanced MMP−NP complexes in solution. A series of colors were obtained with different target DNA1 concentrations: (a) 0, (b) 3.5 μM DNA1, (c) 35 nM DNA1, (d) 350 pM DNA1, and (e) 3.5 pM DNA1.

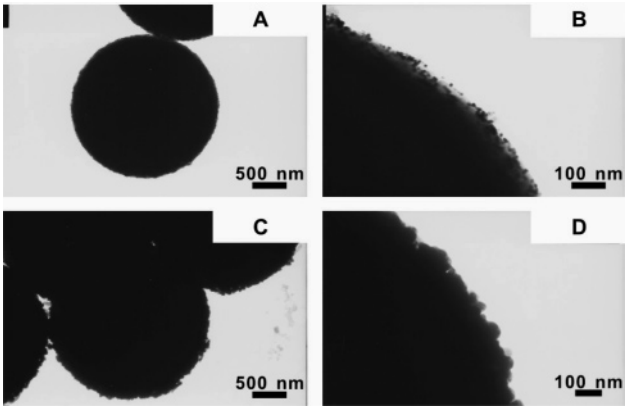

Figure 5. TEM images of MMPs hybridized with Au NPs before (A,B) and after (C,D) silver enhancement.

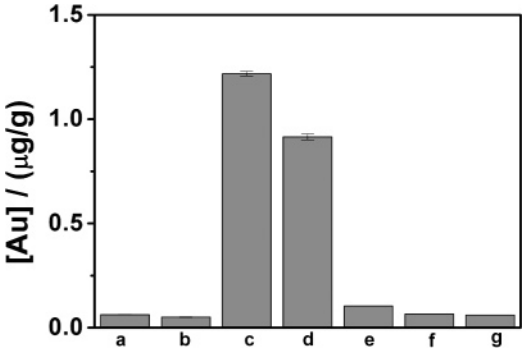

Figure 6. Gold signal obtained by ICP-AES with different target DNA1 concentrations: (a) 0, (b) 3.5 μM DNA4, (c) 3.5 μM DNA1, (d) 35 nM DNA1, (e) 3.5 nM DNA1, (f) 350 pM DNA1, and (g) 3.5 pM DNA1.

to hybridize with more than one MMP in solution led to the reduction of silver ions only on the surfaces of discrete MMPs. Figure 5 shows the TEM images of MMPs hybridized with Au NPs before (Figure 5A,B) and after (Figure 5C,D) silver enhancement. Comparison of these two images allowed us to observe the obvious change in the morphology at the edge of MMPs. The MMP surfaces turned rougher after the silver enhancement. With these results in hand, we were able to then investigate concentration-dependent ICP-AES responses. ICP-AES data of gold element showed a positive correlation with every concentration of target DNA, and the random DNA4 detection also exhibited a signal level as low as the background (Figure 6). The concentration of DNA detected with Au-NP probes could get down to 350 pM. Under the unoptimized conditions that were employed herein, the detection limit by ICP-AES is higher than that by silver enhancement.

Albeit not demonstrated, given the unusually sharp melting profile invariably associated with NP-based DNA detection systems, the single or double mismatch differentiation should be within the reach of our new diagnostic platform, through either thermal or salt stringency wash. Further improvement and optimization of the methodology will provide us with a more competitive sandwich assay.

Conclusion

We have developed an NP-based DNA detection method using ICP-AES as a readout tool. Detection of DNA has been successfully achieved with this sandwich assay format. Further optimization of the experimental variables is anticipated to increase the selectivity and sensitivity. This method transitions ICP detection of biologically significant targets from flat substrate to solution. Our strategy also has many advantages, such as ease of performance, low cost, rapid detection due to faster binding kinetics, and good reproducibility. Although yet to be demonstrated, the most important application of this method is expected in multiplexed DNA detection, via doping different metals into the silica NPs or directly utilizing NPs with different chemical compositions to provide various element signals. With improvement of the instrument, ICP-AES could emerge as a powerful method in DNA diagnostics.

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