DNA Assay

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One-Step Homogeneous DNA Assay with Single-Nanoparticle Detection**

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The development of highly sensitive and rapid methods for detecting DNA is of critical importance in biomedical studies.[1] Currently, the measurements of DNA sequence are predominantly settled by Southern blot[2] or DNA microarray analysis.[3] However, these methods are heterogeneous formats, which require labor-intensive oligonucleotide immobilization procedures, time-consuming hybridization, and multiple steps of washing cycles. To simplify the procedure, homogeneous hybridization methods have been developed, such as fluorescence resonance energy transfer,[4] molecular beacons,[5] TagMan,[6] and fluorescence correlation spectroscopy.[7] All of these methods allow work in homogeneous conditions, but fluorophores have been targeted into the DNA molecules during detection.

In 1997, Mirkin et al.[8] proposed a new colorimetric detection of DNA hybridization by utilizing the distance-dependent optical properties of aggregated gold nanoparticle (AuNP) probes. This method was simple and rapid, but with low sensitivity. Therefore, methods based on the light-scattering properties of AuNPs were proposed to improve the sensitivity of the DNA hybridization assay, including linear light scattering,[9] nonlinear light scattering,[10] and dynamic light scattering.[11] Although the average diameter of aggregates can be detected by these methods, they were limited to monodisperse samples and a scatter-free environment.[12] Recently, a single-nanoparticle (NP) counter technique was reported, which can accurately measure the number of AuNPs, but the size of aggregates cannot be distinguished because of the Gaussian profile of the laser beam.[13] Therefore, the development of a single-particle detection method, which can simultaneously measure the particle concentrations and individual sizes, is of tremendous interest.

Herein, we report a novel method of one-step homogeneous DNA assay using single-NP detection with dual data acquisition by inductively coupled plasma mass spectrometry (ICP-MS). The frequency of the pulse signal is a function of the concentration of AuNP colloids and the recorded peak distribution of signal intensity is a function of size distribution.[14] As illustrated in Figure 1, the hybridization of DNA targets with DNA probes immobilized on the surface of the AuNPs results in the formation of dimers, trimers, or even large aggregates of AuNPs. This polymeric network aggregation leads to decreased concentrations of the whole AuNP population as well as increased individual sizes. These changes can be detected by single-particle ICP-MS (SP-ICP-MS) quantitatively, and thus the amount of DNA is obtained. With this method, concentrations of DNA as low as 1 pM could be achieved. Moreover, the method has another two potential advantages. Firstly, it does not need NPs with optical or electrochemical properties, because the measurement is based on the transient signals of mass to charge induced by the ions originating from the ionization of NPs. Secondly, because of the multielement analysis property of the detector,[15] the method can possibly be used for multiplexed DNA hybridization assay.

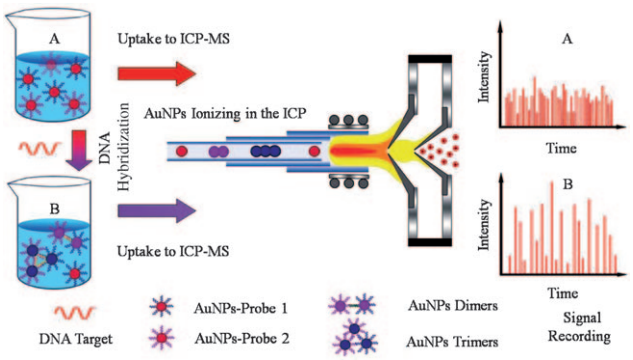

Figure 1. DNA hybridization assay with AuNP probes by using SP-ICP-MS. The first step was to functionalize citrate-protected AuNPs with two sets of single-stranded DNA, probe 1 and probe 2. Then DNA targets were hybridized with AuNP–probe 1 and AuNP–probe 2 in buffer solution. The solution of AuNP aggregates was introduced into the plasma torch by the nebulizer and then AuNPs underwent desolvation, particle vaporization, atomization, and ionization in the ICP zone at approximately 6000–7000 K. Finally, the frequency and intensity of the $^{197}Au^{+}$ pulse signals were recorded by the electron multiplier detector.

To demonstrate the feasibility of this method for DNA hybridization assay, we first conducted an analysis of four types of AuNPs with different sizes and concentrations. As shown in the profile spectra of pulse counts versus time in Figure 2c, only background signals (below 2 counts) were detected in a blank solution; however, transient signals were

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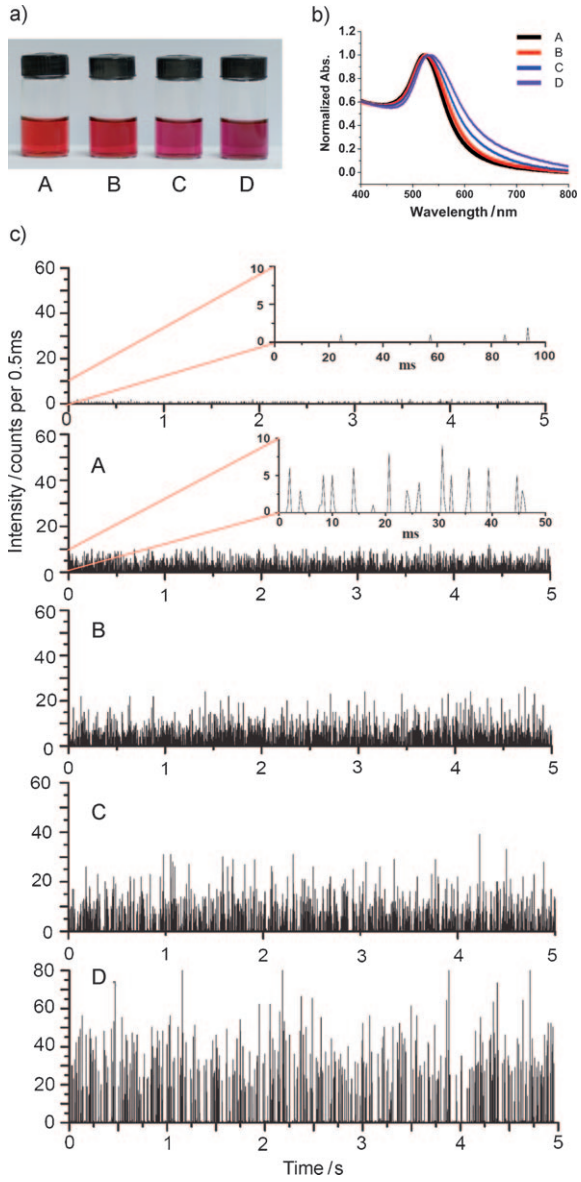

Figure 2. a) Photograph showing the colors of AuNPs with different sizes: A = 24, B = 28, C = 36, D = 45 nm (determined by TEM, see the Supporting Information, Figure S1). b) UV/Vis absorption spectra (normalized) of AuNPs with different sizes. c) SP-ICP-MS profile spectra of a blank solution (top) and solutions of AuNPs with different sizes (A–D). The signal of $^{197}Au^{+}$ ions was recorded per 0.5 ms and 10000 data points were acquired continuously in 5 s, for a solution about 42 μL in volume. The concentrations of AuNPs from A to D were 8.91×10^{6}, 5.09×10^{6}, 3.08×10^{6}, and 2.22×10^{6} particles mL^{-1}, respectively.

obtained from the AuNP suspension that gradually increased with the size. Quantitatively, there was a fine index linear relationship between the intensity and the diameter of the AuNPs (see the Supporting Information, Figure S2a), down to the minimum detectable size of 15 nm. In the study of different concentrations of AuNPs, the frequency of transient signals and the concentrations also showed an excellent linearity (see the Supporting Information, Figure S2b). The reason for this quantitative relationship is as follows. In SP-ICP-MS, each pulse signal corresponds to a single particle, therefore the ^{197}Au intensity of the pulse signal is proportional to the number of Au atoms, which is a function of the size of the AuNPs, and the frequency is proportional to the number of AuNPs. Therefore, SP-ICP-MS would be a powerful tool to quantitatively characterize the behavior of AuNP aggregation.

In the DNA hybridization assay, the DNA target was a 30-base fragment. The 15-base DNA probe 1 and probe 2 were complementary to the DNA target. Citrate-stabilized AuNPs of average size 28 nm were used in this work. As shown in Figure 3, after the addition of DNA targets (10 μL) of different concentrations (0 M, 10 pM, 100 pM, 1 nM) to a 1:1 mixture of two 2.5 μL AuNP probe solutions, different degrees of aggregation were observed by the TEM images. Without DNA targets, AuNPs were monodisperse; however, as the concentration of the DNA targets was increased, dimers, trimers, or even large aggregates of AuNPs emerged gradually. When the concentration reached 1 nM, large aggregates with dozens of AuNPs were observed. Compared with the results shown in Figure 4, we find that the aggregation behavior was quantitatively characterized by SP-ICP-MS. With the formation of the aggregates, the number of total pulse signals in the spectra (Figure 4a–d) decreased gradually from 905 to 817, 710, and 210, while the number of high-intensity signals increased, with the average intensity increasing from 6.843 to 7.368, 8.594, and 16.786 counts. This result reveals that SP-ICP-MS is able to distinguish the concentration of DNA targets by characterizing the degree of aggregation.

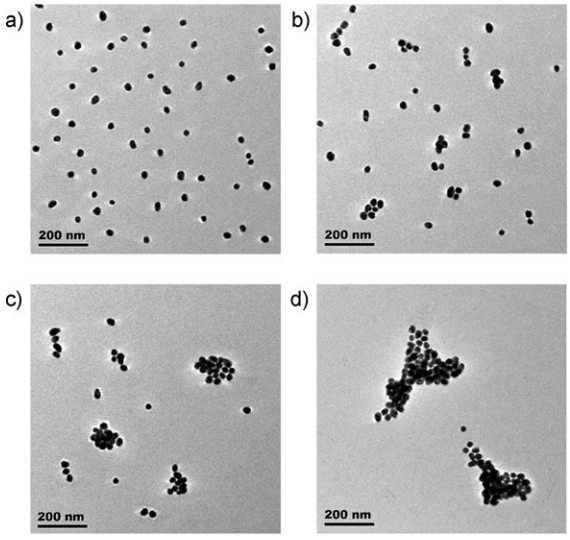

Figure 3. TEM images of aggregate AuNPs after the addition of DNA targets (10 μL) of different concentrations to the AuNP probe solution. This solution is a mixture of two 2.5 μL AuNP probe solutions in 1:1 ratio (v/v). Both the AuNP probe solutions are about 100 pM in concentration. a) Without DNA targets; b) with 10 pM DNA targets; c) with 100 pM DNA targets; d) with 1 nM DNA targets.

The quantitative relationships between the frequency and the average intensity of pulse signals and the concentration of the DNA targets are illustrated in Figure 5. As the aggregates of AuNPs would be too large if the concentration of added

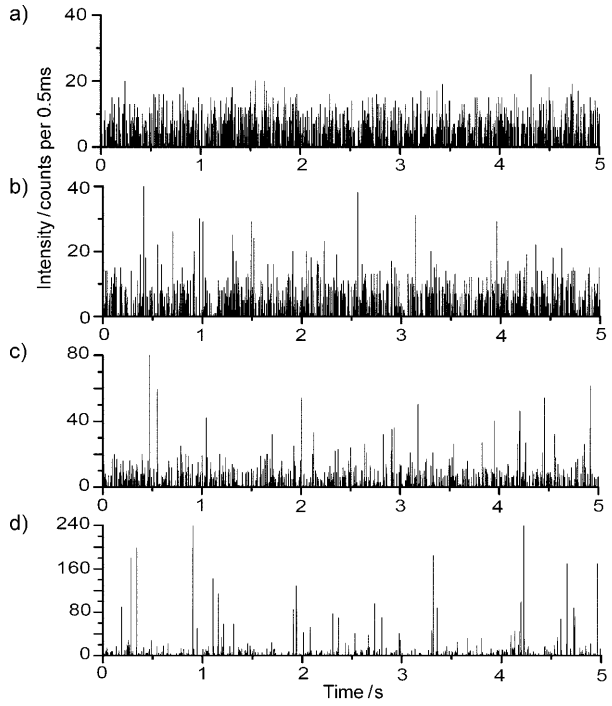

Figure 4. SP-ICP-MS profile spectra of aggregate AuNP solutions after the addition of DNA targets (10 μL) at different concentrations. a) Without DNA targets; b) with 10 pM DNA targets; c) with 100 pM DNA targets; d) with 1 nM DNA targets.

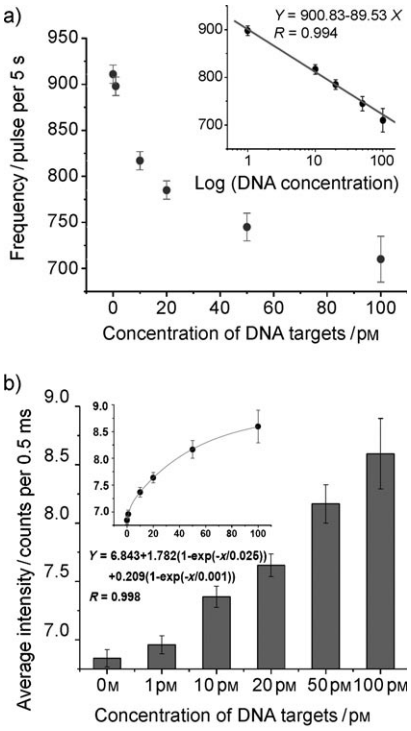

Figure 5. a) Relationships between the frequency and concentration of the DNA targets from 0 M to 100 pM. b) Relationships between average intensity and the concentration of the DNA targets. The dwell time was 0.5 ms in a duration of 5 s. The error bars represent the standard deviation of five measurements.

DNA targets was more than 1 nM, concentrations of DNA targets ranging from 1 to 100 pM were chosen for the quantitative assay. The inset in Figure 5a shows that the frequency and log DNA concentration have an excellent linear relationship; the linear range is three orders of magnitude and the correlation coefficient (R) is 0.994. In Figure 5b, the rate of the increase in the average intensity decreases with the concentration of DNA, so there is no good linear relationship. However, by using polynomial regression it can still be used for the DNA assay.

To further address the selectivity of this new method, an experiment was designed to determine whether the matched DNA targets could be detected in the presence of single base-pair-mismatched DNA targets under the same assay conditions. Three different kinds of such DNA targets were studied and the results are shown in Figure 6. Not only the frequency decrease of single base-pair-mismatched DNA targets but also the degree of intensity increase is much lower than those of the matched DNA targets. Therefore, our method is effective in discriminating single base-pair-mismatched DNA targets from the matched DNA targets.

In summary, we have demonstrated a novel method to detect special sequence DNA targets in one-step homogeneous hybridization solutions with single-NP detection. The DNA targets can be detected at 1 pM by using AuNP probes, which increase the sensitivity by three orders of magnitude over that of colorimetric methods, without any signal amplification process. Compared with other techniques for the detection of AuNPs in homogeneous solutions, such as dynamic light scattering and single AuNP counter techniques,

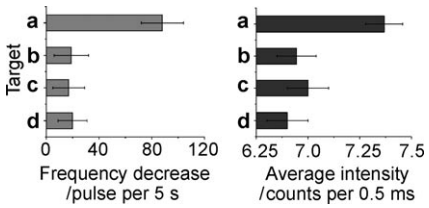

Figure 6. Distribution graphs of frequency decrease (left) and average intensity (right) from matched DNA targets and three different kinds of single base-pair-mismatched DNA targets: a) matched DNA targets; b) 5′-termini one base-pair-mismatched DNA targets; c) 3′-termini one base-pair-mismatched DNA targets; d) middle one base-pair-mismatched DNA targets. The targets were all at a concentration of 10 pM.

ICP-MS can simultaneously differentiate AuNPs of different sizes and provide accurate quantification of AuNPs, and this advantage makes the measurement more reliable and accurate. Additionally, ICP-MS is able to detect NPs without any special requirements for optical and electrochemical properties, so an expanding range of NPs such as biological tags can be applied. Moreover, by choosing a time-of-flight or multicollector analyzer, encoded NPs composed of different elements can be simultaneously analyzed. Therefore, the proposed method may be extended to massively parallel and high-throughput analysis of DNA and other biological molecules.

Experimental Section

The synthesis of citrate-protected AuNPs is described in the Supporting Information. The preparation of DNA-modified AuNP probes and hybridization of AuNP–probe 1 and AuNP–probe 2 with DNA targets were conducted according to the literature.[8,13] AuNPs were functionalized by derivatizing a solution (1 mL, ca. 300 pM) of 28-nm-diameter AuNPs with probe DNA solution of optical density 0.5 (the final DNA concentration was 2–3 μM). After standing for 16 h at room temperature, the mixture was first buffered at 10 mM of phosphate (pH 7). In the subsequent salt aging process, the mixtures were brought to 0.3 M of NaCl by dropwise addition of 2 M NaCl in a stepwise manner three times in 24 h. Finally, the solution was centrifuged to remove the excess DNA (8000 rpm for 15 min). The final concentration of DNA-modified AuNP probes was about 100 pM.

In the hybridization process, AuNP–probe 1 and AuNP–probe 2 (2.5 μL, 100 pM) were mixed in a 1:1 ratio, and DNA targets (10 μL) and one base-pair-mismatched DNA solution with different concentrations were then added. The mixtures were first heated to 70 °C for 10 min and then allowed to cool at room temperature. After 2 h, the mixtures were diluted to 1 mL with 10 mM phosphate (pH 7). In the SP-ICP-MS assay, a solution (10 μL) of mixtures was diluted with ultrapure water (1 mL) and measured by an x series ICP-MS instrument (Thermo Electron Corp., Winsford, UK), equipped with a glass concentric nebulizer and an impact bead spray chamber for aerosol generation and filtration. Before each measurement, the operating parameters of the instruments were optimized by a standard solution of 10 $\mu g L^{-1}$ Au (see the Supporting Information, Table S1). The sequences of DNA used in this experiment were as follows:

Probe 1:	5′-HS-$(CH_2)_6$-$(A)_{10}$-TTG TGC CTG TCC TGG-3′
Probe 2:	5′-GAG AGA CCG GCG CAC-$(A)_{10}$-$(CH_2)_6$-SH-3′
Matched target DNA (a):	5′-GTG CGC CGG TCT CTC CCA GGA CAG GCA CAA-3′
Single base-pair-mismatched DNA (b):	5′-GTG CGC CAG TCT CTC CCA GGA CAG GCA CAA-3′
Single base-pair-mismatched DNA (c):	5′-GTG CGC CGG TCT CTC CCA GGA TAG GCA CAA-3′
Single base-pair-mismatched DNA (d):	5′-GTG CGC CGG TCT CTC GCA GGA CAG GCA CAA-3′

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