Design of one-to-one recognition triple Au nanoparticles DNA probe and its application in the electrochemical DNA biosensor†

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A novel and highly sensitive biosensor for sequence specific DNA detection was constructed based on a strategy of one-to-one recognition reaction between Tri-AuNPs DNA probe signal amplification unit and target DNA.

Developing the methodology of sequence specific DNA detection and recognition units that reveal improved reproducibility and enhanced sensitivity is crucial for gene analysis, tissue matching, pathogenic diseases, and forensic applications and also poses a great challenge for researchers.[1,2] The usual method of detecting a sequence specific DNA target is to use a labeled complementary sequence specific DNA probe. To achieve high sensitivity, different DNA probes have been created with a large range of reporting moieties including fluorescent markers,[3] proteins,[4] radioactive elements,[5] electrochemiluminescent[6] or chemiluminescent compounds,[7] and electrochemical active compounds[8] to facilitate ultrasensitive analysis.

The bio bar codes DNA assay utilizing oligonucleotide-modified Au nanoparticles (AuNPs) for signal amplification in DNA detection has been developed by Mirkin within recent years.[9] In the bio bar codes DNA assay, DNA-functionalized AuNPs have a demonstrated advantage in terms of sensitivity and selectivity over conventional probes in a variety of bio-detection schemes.[2,10] However, the usual bio bar codes DNA assay requires multiple complicated experimental procedures, including the preparation and release of bio bar codes DNA probes from the target-nanoparticle complex, and immobilization and hybridization of the probes on a chip.[11] Sophisticated instruments such as microarrayers and chip-imaging tools are also needed. To overcome these disadvantages, some novel bio bar codes DNA detection methods have been reported recently.[12–14] Moreover, new sensitivity DNA biosensors based on electrochemical detection and multifunctional encoded DNA-Au bio bar codes were also reported recently.[8] But with all these DNA assays based on bio bar codes amplification technology, there exists the one-to-many recognition reaction between signal amplification unit and target DNA. The event of many target DNA hybridized with one AuNPs bio bar codes DNA probe must decrease the sensitivity of DNA detection. Novel strategies based on the coupling of multiple amplification units and processes are still highly desired for meeting the high sensitivity demands of DNA detection.

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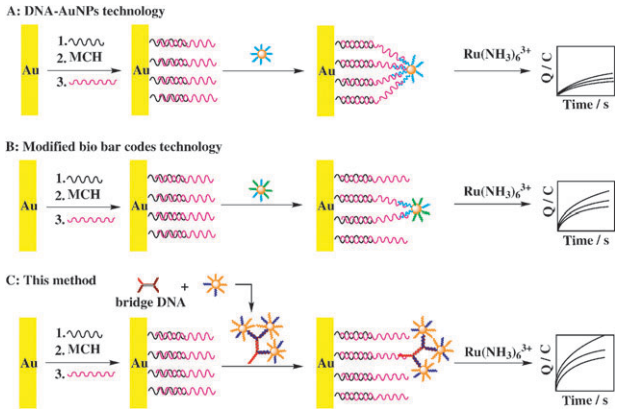
In the present work, a new kind of one-to-one recognition DNA probe based on triple Au nanoparticles (Tri-AuNPs) was reported. The DNA biosensor based on the one-to-one recognition Tri-AuNPs DNA probe was constructed by immobilizing capture DNA on the gold electrode and hybridization with target DNA, which further hybridized with the only one single strand DNA on one terminal of the DNA probe. Electrochemical signals of hexaammineruthenium(III) ($[Ru(NH_3)_6]^{3+}$) bound to the reporter DNA *via* electrostatic interactions were measured by chronocoulometry (CC).

For the preparation of the one-to-one recognition Tri-AuNPs DNA probe, bridge DNA was prepared first with S3 and S4 (see Supporting Information Table S1).† Then two kinds of AuNPs bio bar codes, which were prepared with S6-1, S5 and S6-2, S5, were added to form the one-to-one recognition Tri-AuNPs DNA probe unit (Scheme 1). The one-to-one recognition Tri-AuNPs DNA probe incorporated only one single-stranded DNA acting as recognition DNA and about 486 single-stranded DNA sequences acting as reporter DNA (see Supporting Imformation).† An electroactive complex, $[Ru(NH_3)_6]^{3+}$, which can be bound to the anionic phosphate of the reporter probe DNA strands through electrostatic interaction, served as signal transducer. With the one-to-one recognition Tri-AuNPs DNA probe, the large single-stranded DNA-to-recognition DNA ratio (486 : 1) provided a new strategy of signal amplification for recognizing the target DNA. The successful fabrication of Tri-AuNPs was confirmed by scanning electron microscopy (SEM) (Fig. 1) (see Supporting Information for details).† Through the hybridization action between the bicephalous 18 × 2 bp of S4 and the 18 bp of S3 in the bridge DNA and the conjunctive DNA labeled with the AuNPs, the bridge DNA could be labeled with three gold nanoparticles. It can be seen from the SEM that trimers of gold nanoparticle were formed. This validated our original design that the single AuNP bio bar codes unit can form discrete AuNP bio bar codes assemblies using the bridge DNA as a connector.

To show the excellent utility of the one-to-one recognition Tri-AuNPs DNA probe in ultrasensitive DNA analysis, a sandwich assay was employed, which was shown in Scheme 1. Two DNA strands, namely, S1, S2, and the one-to-one recognition Tri-AuNPs DNA probe were used in this assay. The combined sequences of S1 and one terminal of the bridge DNA on the DNA probe are complementary to the target S2. The thiolated S1 is first immobilized on a gold electrode substrate. S2 and one-to-one recognition Tri-AuNPs DNA probe are then added for hybridization. The detection of the S2 is done by monitoring electrochemical signals of $[Ru(NH_3)_6]^{3+}$ bound to the reporter DNA on the AuNPs



Scheme 1 Schematic diagram for the DNA biosensor fabrication based on one-to-one recognition Tri-AuNPs DNA probe. And the comparison of DNA biosensor fabrication based on AuNPs modified with only one kind of DNA (A: DNA-AuNPs technology), AuNPs modified with two kinds of DNA (B: modified bio bar codes technology), and one-to-one recognition Tri-AuNPs DNA probe technology (C: this method).

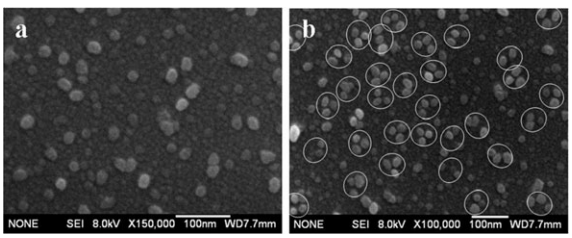


Fig. 1 Scanning electron microscopy (SEM) images of the AuNPs. AuNPs labeled with reporter probe DNA and conjunctive DNA (a); And the AuNPs bio bar codes hybridization with bridge DNA (b).

surfaces left on the electrode surface *via* electrostatic interactions after washing steps.

Subfemtomolar concentration detection limits in DNA analysis have been achieved using the one-to-one recognition Tri-AuNPs DNA probe-based assay. The principle of the signal amplification of the Tri-AuNPs DNA probe is that only one one-to-one recognition Tri-AuNPs DNA probe conjugate hybridizes to one target S2 sequence and one one-to-one recognition Tri-AuNPs DNA probe labeled with three AuNPs were covered with about 486 single-stranded DNA acting as the report DNA. The results showed that the CC intensities of $[Ru(NH_3)_6]^{3+}$ increased with the increase of the concentration of the target DNA ranging from 1.0×10^{-16} to 1.0×10^{-14} M (Fig. 2). The nonlinear function for target DNA was $y = -1.118 \times 10^{-4}x^2 + 0.04029\ x + 0.05320$ (y was the charge, μC; x was the concentration of target DNA, 10^{-16} M; $n = 12$; $r = 0.9987$). On the basis of the control experiments with buffers, the detection limit of this assay was calculated to be 53 aM (3σ). A series of eleven repetitive measurements of 4.0×10^{-16} M target DNA were used for estimating the precision, and the relative standard deviation was 3.8%. It was shown that the DNA biosensor had good reproducibility. This method and some other amplified techniques, which can greatly improve the sensitivity of the DNA assay, are listed in Table S4.†[8a,8e,15]

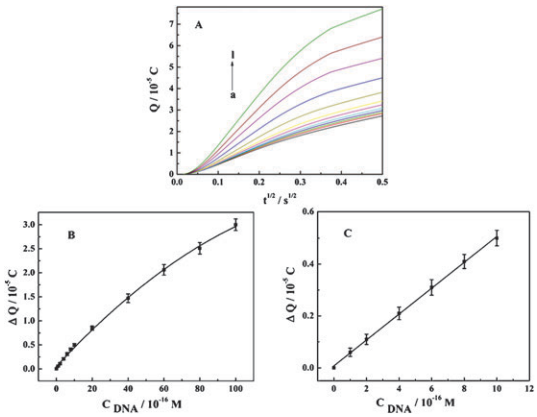


Fig. 2 (A) CC transients of $[Ru(NH_3)_6]^{3+}$ for different hybrids. The concentrations of target DNA from a to I was 0, 1.0, 2.0, 4.0, 6.0 8.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0 $\times 10^{-16}$ M. (B) Calibration curve of the DNA sensor, where the definition of signal is the same as that in (A). The electrolyte was 10.0 mM Tris buffer (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$. Pulse period, 250 ms. (C) is the amplification of the dots 1–7 in (B). The blank was deducted in B and C.

To compare the signal amplification by the one-to-one recognition Tri-AuNPs DNA probe to a one-to-many recognition AuNPs DNA probe, comparison experiments were carried out with the modified bio bar codes method (namely, single AuNP bio bar codes technology, which uses normal AuNPs bio bar codes as the DNA probe) and the DNA-AuNPs amplification technology (which uses DNA modified single AuNP as DNA probe) using $[Ru(NH_3)_6]^{3+}$ as a signal transducer,[8a] respectively. Under optimal experimental conditions, the two probes were hybridized to the target, respectively. The experimental results showed that the one-to-one recognition Tri-AuNPs DNA probe signal amplification technology (this method) demonstrated one target DNA brings one one-to-one recognition Tri-AuNPs DNA probe to the surface, giving approximately a 20-fold improvement in detection sensitivity compared to modified bio bar codes technology (we choose the lowest concentration of the linear range as the comparison standard). And it also increased a 200-fold improvement in detection sensitivity compared to DNA-AuNPs amplification technology[8a] (see Supporting Information, Fig. S3 and S4).†

The improvement on the detection sensitivity of the proposed DNA assay was attributed to two reasons. One reason was that each bridge DNA was labeled with Tri-AuNPs and about 486 single strand DNA on the Tri-AuNPs acted as reporter DNA would improve the sensitivity greatly. Another most important reason was that there was only one single strands DNA which was complementary to the target DNA on each one-to-one recognition Tri-AuNPs DNA probe. This would result in each one-to-one recognition Tri-AuNPs DNA probe hybridizing with only one target DNA rather than many target DNA which must occur in the DNA-AuNPs amplification and modified bio bar codes method[8a] (the principles comparison between the three methods was shown in Scheme 1). In the comparison experiment, many target DNA would react with one reporter NPs probe when AuNPs were labeled with many strand DNA which was complementary to the target DNA.

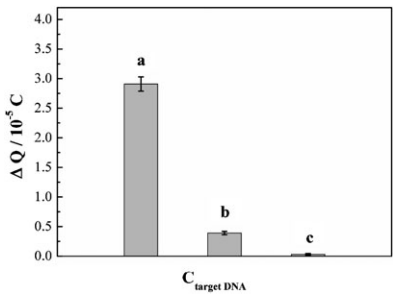


Fig. 3 CC response for different DNA sequences. (a) The complementary target DNA; (b) two-based mismatched target DNA; (c) completely mismatched target DNA. The concentration of target DNA was 1.0×10^{-14} M. The blank was deducted.

This would lower the detection sensitivity. If it was only considered the amplification of three AuNPs, the sensitivity of the presented method should be improved 3-fold compared with modified bio bar codes method. But the experimental results suggested about 20-fold improvement. This validated the design that the one-to-one recognition hybridization reaction between the probe DNA and the target DNA can greatly improve the detection sensitivity.

The one-to-one recognition Tri-AuNPs DNA probe has also provided an excellent capability in discriminating two target DNA sequences that differed by only two bases upon DNA hybridization. The selectivity of the present biosensor was investigated by using one-to-one recognition Tri-AuNPs DNA probe hybridize with the same concentration of complete complementary target DNA sequences, the two-base mismatched DNA sequences and the noncomplementary DNA sequences, respectively. As shown in Fig. 3, a well-defined CC signal of $[Ru(NH_3)_6]^{3+}$ was obtained for the complementary sequences. The CC intensity for two-base mismatched sequences was only about 13.5% of that for complementary DNA at the same concentration, and the noncomplementary sequences showed nearly no response. Although $Ru(NH_3)_6^{3+}$ would also bind to the single stranded DNA, the responses signal would significantly reduced without target DNA. This was because if there was no target DNA the one-to-one recognition Tri-AuNPs DNA probe can not be captured by the capture DNA on the electrode and the responses signal was as low as the blank signal. The presence of the one-to-one recognition Tri-AuNPs DNA probe when the sequences are complementary would produce much greater signals due to the dense oligonucleotide loading on the nanoparticle surfaces. Besides sensitivity and selectivity, reusability is also an extremely important feature for biosensors in practical applications such as clinical diagnoses. It was found that the proposed biosensor could be conveniently regenerated by incubation of modified electrodes in hot water (90 °C) for 10 min, which completely removed hybridized DNA *via* thermal denaturation. More importantly, the CC intensity of the biosensor almost retained 92% intensity of the original performance after three regeneration cycles (see Supporting Information, Fig. S5).†

In summary, a new strategy for the one-to-one recognition reaction between Tri-AuNPs DNA probe signal amplification unit and target DNA was constructed. The sensitivity of the DNA biosensor was achieved through the signal amplification by one-to-one recognition Tri-AuNPs DNA probe. The detection limit of 53 aM of target DNA was obtained. The resulting DNA biosensor exhibited ultrasensitivity, high selectivity, and good reusability as a promise alternative technique for other bioassays. This strategy could lead to the development of efficient tools for rapid, very specific and direct detection of extremely low concentration DNA. With the concept of creating discrete AuNP bio bar code assemblies, multi-AuNPs bio bar codes could also be constructed through the hybridization of branch DNA and nanoparticle-DNA conjugates or the affinity interaction between the branch DNA and nanoparticle-DNA conjugates which could be modified with biotin, avidin and so on. The detection sensitivity would be further improved greatly with the conception of one-to-one recognition action between the signal probe and the target and using the one-to-one recognition multi-AuNPs bio bar codes as a new marker. It also would be used in the design of aptamer biosensor and immunoassay. It is believable that it could make a significant contribution to nucleic acid and protein detection based on the proof-of-concept approach described in this work.

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